Cutaneous manifestations of scrub typhus

A dissertation submitted in partial fulfillment of the rules and regulations for M.D. (Dermatology, Venereology and Leprosy) Branch XX- examination of the Tamil Nadu Dr. M.G.R. Medical University, Tamil Nadu, to be held in April 2013

DECLARATION

This is to certify that the dissertation entitled, "Cutaneous manifestations of scrub typhus" is the bona fide work of Dr. Lydia Mathew towards the M.D. Branch (Dermatology, Venereology and Leprosy) Degree examination of the Tamil Nadu Dr. M.G. R. Medical University, to be conducted in April 2013.

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CERTIFICATE

This is to certify that the dissertation entitled, "Cutaneous manifestations of scrub typhus" is the bona fide work of Dr. Lydia Mathew towards the M.D. Branch (Dermatology, Venereology and Leprosy) Degree examination of the Tamil Nadu Dr. M.G. R. Medical University, to be conducted in April 2013.

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November 7, 2011

Dr. Lydia Mathew
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Sub: **FLUID Research grant project NEW PROPOSAL:**
Cutaneous manifestations in scrub typhus and immunohistochemistry on skin biopsies.
Dr. Lydia Mathew, PG Registrar, Dermatology, Dr. Susanne Pulimood, Dr. Lenin George, Dermatology, Dr. George M. Varghese, Dr. Thambu David, Dr. Sowmya Sathyendra, Dr. Samuel George Hansdak, Dr. OC Abraham, Medicine, Dr. Meera Thomas, Pathology, Dr. John Antony Jude Prakash, Clinical Microbiology

Ref: IRB Min. No 7603 dated 07.09.2011

Dear Dr. Mathew,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Cutaneous manifestations in scrub typhus and immunohistochemistry on skin biopsies" on September 7, 2011.

The Committees reviewed the following documents:

1. Format for application to IRB submission
2. Informed Consent Form and Patient Information Sheet(English, Tamil, Hindi and Telugu)
3. Proforma
4. Cvs of Drs. Susanne A Pulimood,OC Abraham, Leni George, Samuel George Hansdak
5. A CD containing document 1 – 3

The following Institutional Review Board (Ethics Committee) members were present at the meeting held on September 7, 2011 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore- 632002

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Dr. Jayaprakash Muliyil	BSC, MBBS, MD, MPH, DrPH(Epid), DMHC	Academic Officer, CMC	
Dr. Vathsala Sadan (on behalf of Mrs. Rosaline Jayakaran)	M.Sc. (Nursing), RN, RM	Dean, College of Nursing, CMC.	
Dr. Gagandeep Kang	MD, PhD, FRCPath.	Secretary IRB (EC)& Dy. Chairperson (IRB), Professor of Microbiology & Addl. Vice Principal (Research), CMC.	

We approve the project to be conducted as presented.

The Institutional Ethics Committee / Independent Ethics Committee expects to be informed about the progress of the project, any SAE occurring in the course of the project, any changes in the protocol and patient information/informed consent and asks to be provided a copy of the final report.

A sum of ₹ 73,650/- (Rupees Seventy three thousand six hundred fifty only) is sanctioned for 2 years.

Yours sincerely,

Dr. Alfred Job Daniel
Principal & Chairperson (Research Committee)
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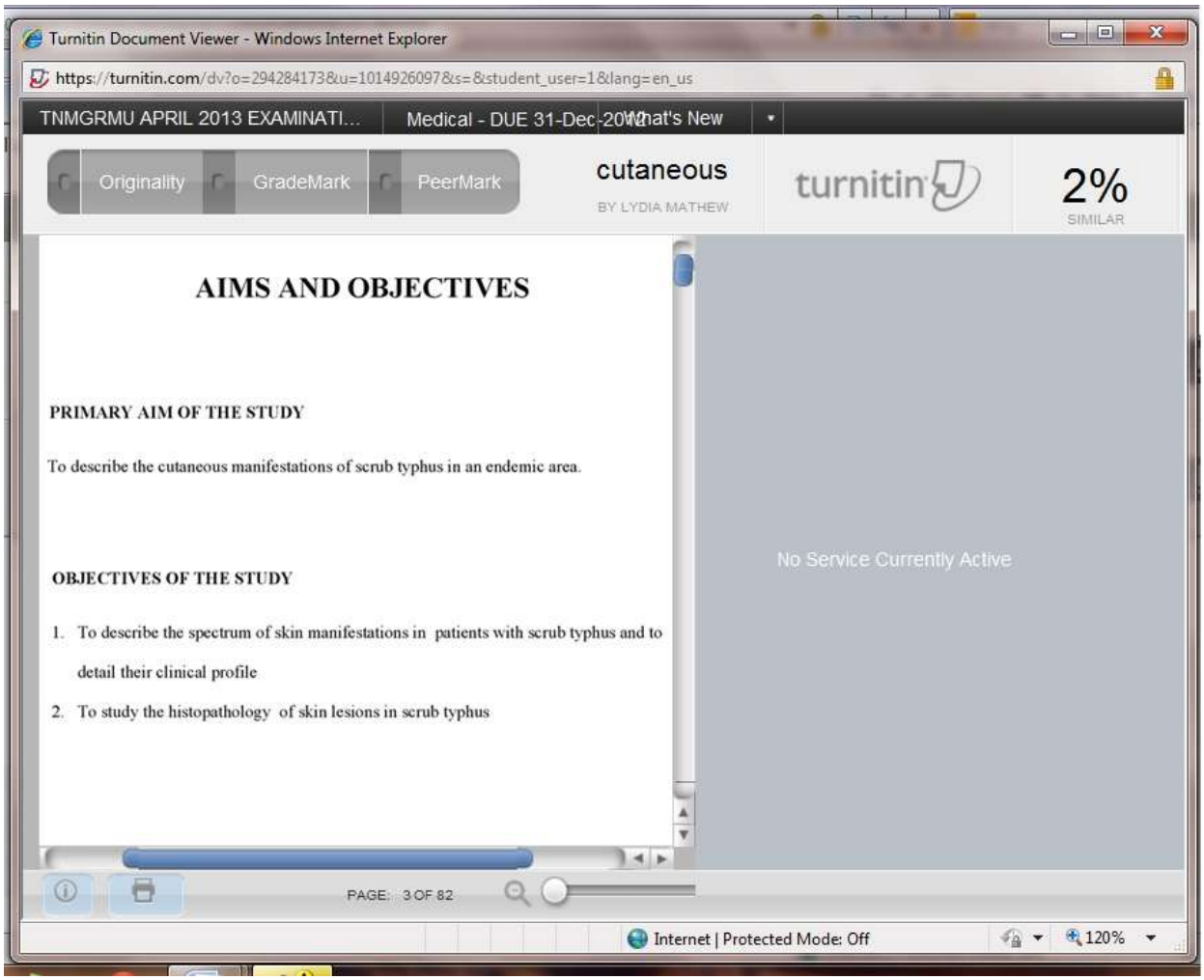
INTRODUCTION Scrub typhus is a rickettsial infection that was discovered in the 19th century in the Orient (1) which was identified as a major endemic area. In the mid 20th century the presence of scrub typhus was observed in India as well. (2) This disease manifests with fever and multisystem involvement resulting in protracted symptoms and death if untreated. The presence of a characteristic eschar helps in a presumptive diagnosis of scrub typhus. Specific antibiotic therapy if initiated results in dissolution of symptoms within 48 to 72 hours. This dramatic therapeutic response makes it imperative to diagnose this infection early. This requires a careful and thorough cutaneous examination...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OBJECTIVES OF THE STUDY
1. To describe the spectrum of skin manifestations in patients with scrub typhus and to detail their clinical profile
2. To study the histopathology of skin lesions in scrub typhus
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This dissertation would be incomplete without expressing my gratitude to all those involved in its conception and completion.

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Above all I thank God Almighty who made all this possible.

INDEX

ABSTRACT

TITLE OF THE ABSTRACT : Cutaneous manifestations of scrub typhus

DEPARTMENT : Dermatology, Venereology and Leprosy

NAME OF THE CANDIDATE : Dr. Lydia Mathew

DEGREE AND SUBJECT : M.D Dermatology, Venereology and Leprosy

NAME OF THE GUIDE : Dr. Susanne Pulimood

SUMMARY

OBJECTIVES:

To describe the skin manifestations, clinical profile and histopathology of skin lesions in patients with scrub typhus.

METHODS:

A cross-sectional, observational study was performed in a tertiary hospital in Vellore, an endemic area for scrub typhus. Among adult patients with a febrile illness of 5 – 28 days duration with either a positive serum scrub typhus IgM ELISA or with an eschar, 84 patients

were recruited from October 2011 to October 2012.Eschar size, distribution of skin lesions, demographic details and clinical features were recorded. The eschar was biopsied for histopathological examination. Data was analysed using Chi-square test, Fisher's exact test and Student's t-test.

RESULTS:

Patients with scrub typhus from Vellore constituted 58/84 of the study patients while the remaining were from the neighbouring districts of Tamil Nadu and Andhra Pradesh. The mean age was 44.4 ±17 years. Agricultural labourers formed the major group with 51.2 % (43/84). Average duration of fever at presentation to hospital was 9.9±4.4 days. Eschars were found in 85.7 % (72/84) of the study patients, one of whom had a concomitant rash.

Maximum number of eschars; 45.8% (33/72) were located over the trunk. There were more eschars found over the anterior aspect of the body ; 87.5 % (63/72). Discrete ulcers without the overlying necrotic scab were noted in 21/72 patients and were mostly over the inner aspect of thighs (7/21). There was no significant difference in gender-wise distribution of eschar. The mean eschar size was 7.1±4.5mm. Eschars less than 5mm were seen in 15/72 (20.8 %) patients. The largest eschars were over the groin and neck which was statistically significant ($p<0.05$).Absence of eschar did not correlate with severe scrub typhus. Regional lymphadenopathy was seen in 13.8 % (10/72) and generalized lymphadenopathy in 6% (5/84), Most patients responded to specific antibiotic therapy. However in 4/ 67 patients defervescence took more than 72 hours. Histopathological examination of the eschar revealed vasculitis in 32/43, vasculopathic reaction in 8/43 and non-specific inflammation in 3 of which had one had peri-adnexal inflammation. Of the patients with vasculitis 75 % (24/32) had lymphocytic vasculitis, 6.25% (2/32) had leukocytoclastic vasculitis in, 18.75% (6/32) had mixed vasculitis,

Panniculitis was seen in 27.9% (12/43) specimens which were mostly lobular ; 58.3% (7/12), 3/12 (25%) had septal panniculitis whereas 2/12 (16.6%) had septal and lobular panniculitis. Rare features like intraneural inflammation was seen in 2/43 , granulomatous inflammation including a lipogranuloma was seen in 2/43 , interphase changes in 2/43 with sub-epidermal cleft in one of them were seen.

CONCLUSIONS: Our study population of patients had a higher occurrence of eschar but a low frequency of rash. Eschars may not always present with a necrotic scab. Larger eschars were located over the neck and groin. Eschars were most frequently located over the trunk. Absence of eschar was not associated with risk of complications. Leucocytosis was found to be more in patients with complications. Delayed defervescence with specific antibiotic therapy of fever was noted in few patients. Lymphocytic vasculitis and panniculitis were the common histopathological findings in eschar biopsies.

INTRODUCTION

Scrub typhus is a rickettsial infection that was discovered in the 19th century in the Orient (1) which was identified as a major endemic area. In the mid 20th century the presence of scrub typhus was observed in India as well. (2) This disease manifests with fever and multisystem involvement resulting in protracted symptoms and death if untreated.

The presence of a characteristic eschar helps in a presumptive diagnosis of scrub typhus. Specific antibiotic therapy if initiated results in dissolution of symptoms within 48 to 72 hours. This dramatic therapeutic response makes it imperative to diagnose this infection early. This requires a careful and thorough cutaneous examination in patients with acute undifferentiated febrile illness from endemic areas.

Scrub typhus is caused by *Orientia tsutsugamushi* which is transmitted by the larval stage of the trombiculid mite known as 'chigger'. Cutaneous features characteristic of scrub typhus is the eschar which is an asymptomatic necrotic scab overlying an ulcer that is preferentially located over the damp areas of the body such as the flexures. This is the site of the chigger bite. Patients may also present with a petechial or a maculopapular rash.

Vellore was first identified as an endemic area in the late 20^{th} century. (3) However the studies on scrub typhus have primarily looked at clinical manifestations, morbidity and mortality of hospitalised patients. Maculopapular or petechial rash which is a part of rickettsial infections as documented from Far East Asia has been rarely observed in these parts. There has not been any organized data on the cutaneous manifestations of scrub typhus from this large endemic area in

South India. Hence we conducted this study to identify and study the cutaneous features of this zoonotic infection.

AIMS AND OBJECTIVES

PRIMARY AIM OF THE STUDY

To describe the cutaneous manifestations of scrub typhus in an endemic area.

OBJECTIVES OF THE STUDY

1. To describe the spectrum of skin manifestations in patients with scrub typhus and to detail their clinical profile
2. To study the histopathology of skin lesions in scrub typhus

REVIEW OF LITERATURE

INTRODUCTION

Scrub typhus is a zoonotic infection caused by the rickettsial organism *Orientia tsutsugamushi*. It manifests as an acute febrile illness with a pathognomonic eschar and other less characteristic cutaneous lesions. It is complicated by systemic features involving major organ systems which can result in death if untreated.

HISTORY OF SCRUB TYPHUS

The term 'scrub typhus' was coined by Fletcher in 1927 wherein 'scrub' refers to the vegetation where the mite is found and 'typhus' is derived from the Greek word 'typhos' which means stupor (1) probably referring to the morbid illness this infection can produce in its victims. This disease was also referred to as mite-borne typhus, chigger-borne rikettsiosis, tropical typhus and rural typhus. The earliest historical report of scrub typhus dates back to third century A.D from China which was documented in a report by Li Shin-Chen in 1885. It described a patient whose disease was attributed to 'red insects' with symptoms suggestive of scrub typhus. There were various reports of this disease in the 19th century from Japan, as early as 1810 when Hashimoto described it in this country. In Japan scrub typhus was known by various names such as Japanese river or flood fever, *akamushi* (red mite) fever, *shima-mushi* (island insect disease) and tsutsugamushi disease. (1) (5) Subsequently this infection was detected in other parts of Asia including China, Indonesia, Taiwan, Philippine, Federated Malay States as Malaysia was known as then, Vietnam, Korea, Pescadores Islands as well as in the Pacific area and in Australia. (5)

During the Vietnam War and World War II scrub typhus posed a significant health problem among military personnel being second only to malaria. (5) Once scrub typhus infection was identified vigilant measures were undertaken in these endemic regions which led to a decline in the number of cases. However this rickettsial infection appears to have made a resurgence with reports from previously undescribed parts of the world in the last few decades.

ETIOLOGY

In the 1920's the causative organism was identified as a rickettsial organism termed Theileria *tsutsugamushi* and thereafter *Rickettsia orientalis.* (6) It was later renamed Rickettsia *tsutsugamushi* and thereafter *Orientia tsutsugamushi* by which it is now known. (7) Hashimoto coined the term *Tsutsugamushi* derived from the Japanese word '*tsutsuga'* which means small and dangerous and "*mushi"* which means creature. (1)

They are obligate, intracellular, non-motile, Gram negative bacteria (though it does not stain well) and measure 0.3-0.5 by 0.8-1.5 µm. They appear as coccobacilli or at times rod-like. They may stain bipolarly and appear like diplobacilli. They have a cell membrane and a cell wall and can also be stained by Giemsa or Gimenez method. (8)

More than 20 strains of *Orientia tsutsugamushi* have been identified such as Karp, Kato, Gilliam, Kuroki and Kawasaki (9), Boryoung, Shimokoshi, Kawasaki-like, and TA763-like with the Karp strain being the most commonly identified. The 56-kDa protein on the cell surface of the organism results in type specific antigenicity. (7)

THE MITE (VECTOR)

Trombiculid mites belonging to the *Leptotrombidium* species serve as vectors for transmission to humans. The various species implicated are *Leptotrombidium akamushi* (formerly *Trombicula akamushi*), *L. deliense, L. akamushi, L. pallidum, L. scutellare, L. arenicola* and many others.

L. deliense is the vector that has been responsible for outbreaks of scrub typhus in India. (10) Studies have shown that *L. dihumerale, L. subintermedium* and *Schoengastiella ligula* also serve as vectors in India. (11)

The mites are found in areas with humidity and have the ability to survive long periods in water reflecting outbreaks of the disease during the rainy season, following floods and the endemicity of this infection along river banks.

A trombiculid mite has 4 stages in its life-cycle i.e., egg, larva, nymph and adult. The mite requires ingestion of tissue fluids for development from the six-legged larval stage to the eight-legged nymph. (12) Humans acquire infection with the bite of the larval stage of the trombiculid mite otherwise known as chigger. The larva feeds on the host once in its lifecycle. The nymph and the adult mite are found free-living in the soil. Studies also show transtadial and transovarial transmission in trombiculid mites which refers to the transmission of the rickettsial organism throughout its life-cycle, indicating that the mite can act as a reservoir of infection as well. (13) **(Fig.1)**

Wild rodents of the genus *Rattus* (14) are the main reservoir of this organism. Many other mammals and ground birds have also been found to be natural reservoirs. Human are accidental

hosts. Ecological areas favourable to the growth of the mite and transmission of rickettsiae are known as ‘mite islands’ or ‘typhus islands’.(15)

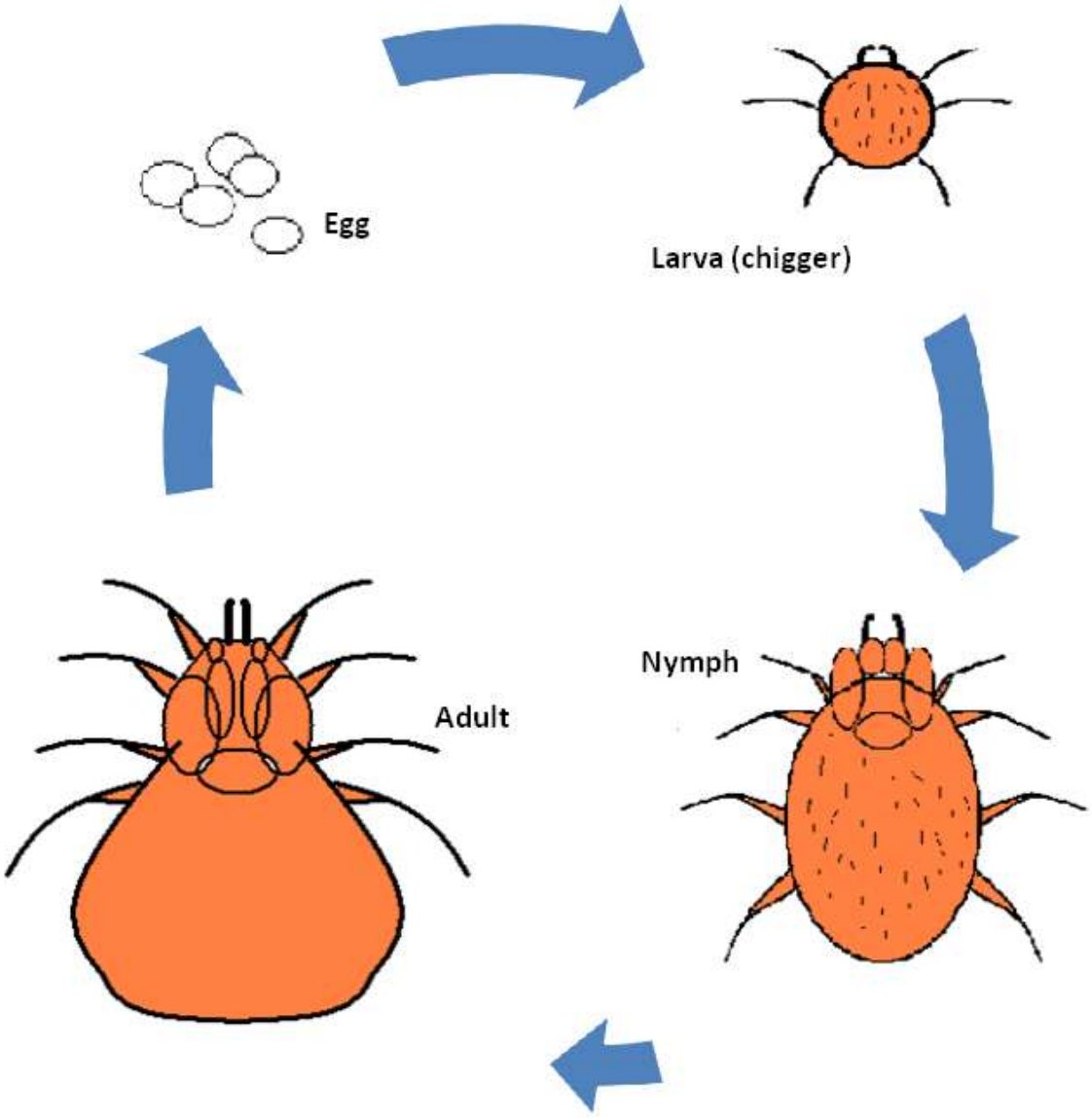


Fig. 1: Life cycle of the trombiculid mite

EPIDEMIOLOGY

Nearly one million cases of scrub typhus occur every year worldwide. (16) Scrub typhus has been found to occur in areas with tropical climate frequented by monsoons. (17) Earlier reports from India are from the hilly terrain of Kashmir, Assam, Eastern and Western Ghats. It was found to occur during the cooler months of the year from August through January with peak occurrence in September correlating with the rainy season as reported in a study from Tamil Nadu. (18) There are reports from sandy and semiarid areas as well. In tropical countries sporadic cases can occur the rest of the year as well whereas in temperate climates scrub typhus occurs seasonally. The initial reports from China were during the summer, thereafter cases were noted to occur in autumn and winter. (19) It has also been reported in spring time (April) from China. (20) Variations in climatic conditions influence transmission of the disease by the vector. Unusual occurrences have been reported from temperate climates.

It classically occurs in terrain with 'fringe habitats' , which refers to the landscape bordering fields and forests , narrow stretches of land along streams, river banks, clearings within jungles with growth of scrub vegetation . It commonly occurs in agricultural labourers, people who stay or work near wood piles, logging, involved in clearing vegetation and has been reported among workers in oil palm, rubber estates (5) , tea estate workers. Travel to endemic areas (21) (22) (23), ecotourism, increasing involvement in outdoor activities like gardening, trekking, hiking, rafting, camping predisposes to acquiring this zoonotic disease. Military corps are also at risk for acquiring this infection.

It occurs commonly in eastern and southeastern Asia and the western Pacific region from 20° south of the equator to about 40° north latitude . This constitutes a triangular area bound by

northern Japan and far east Russia in the east, Pakistan and Afghanistan in the west and northern Australia in the south known as the 'tstsugamushi triangle'**(Fig. 2)**. (15) It is endemic in Korea (24) , Japan (25) (26), China (19) , Thailand (27), Taiwan (28), Vietnam (29) , Indonesia (30), Philippines (31) , Pacific Islands (32), Malaysia (33), Australia (34), New Guinea (35), Cambodia (36), Hong Kong (37), Myanmar (38), Pakistan (39), former U.S.S.R (40), certain areas in India and new reports of occurrence in Sri Lanka. (41) Serological evidence in mammals was reported in Iran, Nepal.

Fig. 2: Tsutsugamushi triangle

THE INDIAN SCENARIO

Scrub typhus has been reported from India at different periods. Many soldiers along the Indian border have succumbed to this infection during World War II and the Indo-Pakistan conflicts of 1965. (2) Subsequently the incidence had reduced presumably due to the widespread use of empiric antibiotics for the treatment of febrile illnesses, increasing use of insecticides and changing lifestyle practices involving fewer outdoor activities. In India, resurgence of this infection was detected serologically from Jammu and Kashmir and Rajasthan reported in 1978 (42) and from patients with pyrexia of unknown origin from Southern India (Tamil Nadu) from 1996 to 1998. (3) Thereafter many outbreaks have been reported from the Himalayas (43), North-eastern states (44), Kerala (45) (46), certain areas in Tamil Nadu (18) (47) (48), the sub-Himalayan belt from Jammu (49) to Nagaland, Manipur (50), Himachal Pradesh (51) (52), Haryana (53), Sikkim and Darjeeling (54), Karnataka (55), Uttarakhand (17), Andhra Pradesh (47) (56), Pondicherry (57) (58), outskirts of Pune (59), Chennai (60). Serological evidence of infection have also been documented from Delhi. (61)

In Tamil Nadu clinical cases of scrub typhus has been reported from Vellore, Chennai and from the nearby Union Territory of Pondicherry. In patients with acute febrile illness, serological evidence of rickettsial infection including scrub typhus by positive Weil-Felix test was obtained by Kamarasu et al in 115/ 306 patients (37.6 %) in the year 2004 and in 89/964 patients (9.2 %) in the year 2005 across 8 districts of Tamil Nadu including Vellore, Thiruppathur, Tiruvannamalai, Cheyyar, Erode, Coimbatore, Udhagamandalam and Thiruvallur. However the data of positive scrub typhus and the exact clinical profile of these patients were not available.(62)

PATHOGENESIS

Following inoculation into the skin *O. tsutsugamushi* disseminates to reach target organs. It has a predilection for macrophages and endothelial cells. It bypasses the phagolysosomes and replicates within the cytoplasmic milieu. Studies have demonstrated the activation of transcription factor activation protein 1 that induces the gene that encodes macrophage chemo-attractant protein 1 in endothelial cells. (63) Regional lymphadenopathy indicates lymphatic involvement. The organism has been detected in peripheral white blood cells as well. (64) Hence it has been concluded that dissemination of the pathogen can occur through the haematogenous as well as the lymphatic route. It results in a vasculitic process in many organ systems that can culminate in multiorgan dysfunction. Studies in patients with HIV-1 have shown that co-infection with scrub typhus suppressed HIV-1 viral replication however these suppressive factors have not been elucidated. (65) Osteopontin is a phosphorylated acidic glycoprotein that acts as a pro-inflammatory cytokine expressed by macrophages, endothelial cells and a few other tissues. Studies in humans have shown the relationship between bacteremia and the levels of IL-10 and pro-inflammatory cytokines. (66)

CUTANEOUS MANIFESTATIONS

ESCHAR

The site at which the chigger bites forms an eschar. (67) Patients are usually not aware of having been bitten by the chigger though few patients report the sensation of being bitten, retrospectively. The eschar itself is asymptomatic and often not noticed by the patient. Following

the bite there is formation of a papule followed by a vesicle which ulcerates and thereafter gets covered by an eschar. (12) An eschar is necrotic tissue that forms a black scab. Underlying the eschar there is ulceration. According to Kim et al the typical eschar ranges from sizes 5 to 20 mm in diameter and is surrounded by a rim of erythema. They have been morphologically likened to burns caused by cigarette stubs. They are usually single but may rarely be multiple. Double eschars were reported in a patient from Turkey (68) and reports from Korea document upto three eschars in a patient, one each on the face, chest and the abdomen as well. (12) There is one report of a patient, from Korea, with numerous eschars over the face, who had a concomitant maculopapular rash over the rest of his body. However a histopathological diagnosis was not available. (69) Allen et al in one of the early descriptions of cutaneous lesions in scrub typhus describes an eschar to have a maximum diameter of 5 mm in size with an erythematous areola of the same size. He reports two patients with double eschars. (70)

They are usually located at warm and moist areas of the human body like the flexural areas and the genitalia. They have been reported to occur in areas of the body such as axillae, inframammary areas, intermammary areas, infragluteal crease, infra-abdominal crease, groins, cubital fossae, popliteal fossae, medial aspect of proximal extremities, genital area including the scrotum, penile shaft (67), labia minora (71) and other areas of the vulva, perineal area, neck and rare reports of occurrence on the frontal area of the scalp, external auditory canal, wrist joint.(67) In flexural areas and the perineum which are usually damp and warm they often present as a discrete asymptomatic shallow ulcer with a purulent base and peripheral erythema without the overlying eschar. (12) Secondary infection of the eschars has rarely been reported (72) though possible since many of them are present over the groins and axillae which may prolong the presence of an eschar. (70)

There is a significant difference in location of the eschar between the sexes as reported in a study of 162 patients of scrub typhus with eschar from Korea. In males eschars tend to be more common within 30 cm below the umbilicus while in females they are seen mostly over the anterior chest. This difference probably reflects changes in clothing patterns between males and females leading to different areas of occlusion and dampness. The moist environment helps the chigger attach itself onto humans for a meal transmitting infection in the process. In this study 92.04 % (n=176) of scrub typhus patients were detected with the characteristic eschar. In deeply pigmented skin eschars could be missed as noted in paediatric Thai patients. (12)

The occurrence of eschars has a variable frequency in different endemic areas. 23.1 % from Taiwan, 30.3% from Lao (16), 46 % to 92 % from Korea (12), 67 % to 88.5% from China (73) (19) and a recent large scale study of 1722 patients with 86.3% (74) , 87% from Japan (75). Observation from previous studies from Southern India suggests the presence of eschar in about 46 % to 50% of patients with scrub typhus (47) (58) and a smaller study from Karnataka with a frequency of 6 % . (55) A study of 21 cases from Himachal Pradesh showed rash in 10 % and eschar in 10 % of patients. (52)

The incubation period is from 10 to 12 days. The presentation of eschar in a patient is usually concurrent with systemic symptoms. Earlier studies have shown that it may take upto 3 to 4 weeks for an eschar to develop. (70) 3

Absence of eschars has been associated with severe and complicated scrub typhus in studies. Kim et al demonstrated this in a case-control study of patients from Korea (76) and Lee at al demonstrated the same in another study of 302 patients from Korea. (77)

Lymphadenopathy, both regional and generalized, has been noted in studies. A large study with 416 patients from Japan reported lymphadenopathy in 51% of patients with three-fourths of them being regional lymphadenopathy. (78) Tender generalized lymphadenopathy has also been reported in many cases. (79) There was a report of severe cervical tender lymph node swelling simulating deep neck infection in one patient. (80) In this patient an eschar was found over the scalp on careful examination. Certain authors have suggested careful examination of lymph nodes to identify the location of eschar. Examination of cervical lymph nodes for the head and neck area, axillary lymph nodes for the upper trunk and upper limbs and the inguinal lymph nodes for the lower limbs and perineal area need to be assessed. (12) There is a rare report of the occurrence of Kikuchi-Fujimoto's disease in cervical lymph nodes in a patient with scrub typhus who also had serological markers for lupus erythematosus. This patient's symptoms recovered on treatment with doxycycline.(81)

Eschars are also found in other rickettsial infections like rickettsial pox, Mediterranean spotted fever, Queensland tick typhus, African tick-bite fever and other illnesses like spider bites and cutaneous anthrax. (82)

Animal models have been developed to study the cutaneous lesions at the site of inoculation of the organism. Intradermal inoculation of the antigen into Cynomolgus monkeys resulted in the development of an eschar in 2 out of 6 animals while the rest developed dusky plaques along with regional lymphadenopathy. Histopathological features were similar to eschars found in humans. (83)

RASH

Scrub typhus may also present with a maculopapular rash that usually ranges from 3 to 10 days that can develop in the second week of illness. (5) Reported frequencies are in the range of 34 % to 71 % from Malaysia, 68.6 % (74) to 76 % from China .(73) Maculopapular rash has not been reported as commonly in the Indian subcontinent. In Southern India the frequency of rash is about 6% (a study from Tamil Nadu) to 13 % (Karnataka) in patients with scrub typhus which has been described as a maculopapular to petechial rash or palpable purpura.(47) (55) They are often purpuric or petechial in nature due to associated thrombocytopenia as noted by Allen et al. Microvascular occlusion with purpura fulminans as reported in spotted fever has not been reported in scrub typhus.

An enanthem was noted by Allen et al over the soft palate but sparing the buccal mucosa.(70)

In children a smaller frequency of eschars was reported in studies from Thailand with 7%. In a series of 27 children from Southern India eschars were found in 4 and rash in 6 patients. (84) There are case reports from Vellore reporting absence of eschar in children. (85) However studies from China report higher frequencies of both eschar and rash as in one study involving 56 children with eschars in 54 and rashes in 55 children (86) and another study with eschars in 84 % and rash in 91 % (n=70) .(87). Eschars were reported in 12 of 15 children with scrub typhus from Taiwan. (88)

OTHER CLINICAL FEATURES

Scrub typhus is a potentially fatal illness. Fever, chills, headache, malaise, cough, dyspnoea, abdominal pain, nausea, vomiting, diarrhea, acute abdomen (89), may be the presenting symptoms.

Fever is intermittent and high grade in nature. In a study done in a tertiary care centre in South India (n=398) scrub typhus constituted 47.5 % of cases of acute febrile illness (90).
Patients can present with relative bradycardia which is defined as less than 10 heart beats per minute for 1 degree rise in temperature as has been noted in 53/ 100 patients in a study from Thailand. This has been observed in infections with other intracellular gram negative and viral organisms though its significance is not clear. (91)

Respiratory symptoms occur in 20 to 72 % of patients. The patient may be found to have bronchitis, interstitial pneumonitis leading onto acute respiratory distress syndrome (ARDS).

A study from Tamil Nadu showed the presence of ARDS in 24.9 % (n=189). Imaging reveals bilateral reticulo-nodular opacities, septal lines, hilar and mediastinal lymphadenopathy and rarely consolidation, pleural effusion, pulmonary edema. (47)

Cardiac involvement includes cardiomegaly due to myocarditis and pericarditis. (92)

Acute renal failure can occur due to systemic vasculitis, decreased perfusion secondary to shock and possible direct invasion of renal tubular cells. Haematuria (93) and proteinuria can occur. (94)

Deranged liver function test is a common feature which includes transaminitis, elevated alkaline phosphatase and total bilirubin as documented in 29 of 30 patients in a study from Taiwan. (95) Vasculitis of the gastrointestinal tract , gastrointestinal haemorrhage (96), thickening of the gall bladder wall, acute cholecystitis complicating gall stones (97), acalculous cholecystitis and pancreatitis (98)(99), pancreatic abscess (61) , splenomegaly, splenic infarction and rare occurrences of atraumatic hemoperitoneum (100) have been reported.

Neurologic symptoms develop due to underlying lymphocytic meningitis, encephalitis (101). Uncommon forms of neurologic involvement includes subdural hematoma (102), cerebral infarction, demyelinating sensori-motor polyneuropathy (103), Guillain - Barre syndrome (104), acute transverse myelitis (105), brachial plexus neuropathy probably related to axonal degeneration and a case report of mononeuritis multiplex involving the median nerve and the ulnar nerve. Mononeuritis may be secondary to a vasculitic process in the peripheral nerve induced by the pathogen; however a tissue diagnosis of the same was not available.(106) A recent case report from Taiwan described a patient with myoclonus and features of Parkinsonism with serological evidence of scrub typhus whose symptoms improved on treatment. (107)

Reports of associated conjunctival suffusion (29) (108) and increasing reports of acute reversible hearing loss (109) have been described.

Septic shock, disseminated intravascular coagulation (75) (110), haemophagocytosis (111) and sometimes even multiorgan dysfunction are the more serious complications. (112)

Mortality is often secondary to cardiac failure, pneumonia, and encephalitis. Concomitant infection with leptospirosis (113) (114) and dengue has been reported.

Differences in clinical features from various endemic areas could be attributed to the varied genotypes of *O. tsutsugamushi* as observed in a study from Korea .This study reported a higher frequency of eschar and rashes with the Boryoung strain than with the Karp strain.(115)

MORBIDITY AND MORTALITY

In a prospective study with 153 patients from Korea, difference in strains or delay in treatment with antibiotics did not affect severity. Factors responsible for severe disease were identified as old age, absence of eschar, leukocytosis, hypoalbuminemia, underlying conditions like diabetes mellitus, cerebrovascular disease, pulmonary and hepatic diseases and serum osteopontin levels > 100 ng/mL whereas anemia (</= 10 g/dL and CRP levels > 10 mg/dL reflected current severity. (116) In another study with 208 patients from Korea, severe clinical features were found in 42.8 % (89/208) of patients. (76)

In the earlier days when fewer antibiotics were available, reported mortality rates ranged from 30 to 50 % (13). A study from a tertiary care hospital set in an endemic area in South India detected a mortality rate of 12.2 % (n=189) in hospitalized patients. It identified metabolic acidosis, acute respiratory distress syndrome, altered sensorium and shock as independent predictors of mortality. (47)

Re-infection is possible since there are multiple strains of *Orientia tsutsugamushi.* Immunity to the same strain (homologous) lasts for 1-3 years whereas heterologous immunity lasts only for a few months. (117) Reactivation of the disease as seen with *R. prowazekii* has not been known to occur.

LABORATORY DIAGNOSIS

Various methods have been described which involves

a) Isolation of the organism
b) Serology
c) Tests on cutaneous lesions of eschar and rash.

ISOLATION OF THE ORGANISM

Earlier methods included smears from infected tissue often obtained at autopsy, stained by Giemsa stain which would only occasionally reveal the organism.

Inoculation of infected tissue into animals like white mice followed by isolation of the organism would take two to three months for a diagnosis due to variable virulence in mice. Isolation from yolk sac of developing chick embryo often had problems with contamination. (5) Culture of these organisms also poses a risk to laboratory personnel requiring biosafety level 3 facilities and is a laborious process.

SEROLOGY

Confirmation of scrub typhus in routine clinical practice has been based on serological assays considered as the gold standard for diagnosis.(118) The first test developed was the Weil-Felix test which demonstrates cross-reactivity between rickettsial antibodies and the *Proteus* antigen OXK. However it is found to be positive only in 40 to 60 % of scrub typhus patients. False positive results occur with urinary tract infections due to *Proteus* species, leptospirosis and relapsing fever.

Other methods include analyzing specific antibody titres by indirect imunofluorescence, indirect immunoperoxidase, IgM ELISA assays. A four-fold increase in antibody titres of serum from the acute and the convalescent stages is diagnostic. Their sensitivity is between 90.6 - 100% (119) (120) and specificity between 96 - 100% .(119) (121) (122) The disadvantage of these tests is the time required to make a definitive diagnosis as it may take from several days for a rise in titres. (123) Complement fixation tests are not commonly used. Latex agglutination techniques have also been developed.

In vitro assays of cell mediated immunity (lymphocyte transformation tests, production of mediators) in scrub typhus have also been developed as diagnostic methods.

PCR of the buffy coat has a sensitivity of 82.2% and a specificity of 100%. (124) PCR of the skin lesions of scrub typhus have also been developed as a diagnostic tool. A similar accuracy has been reported with the eschar i.e., sensitivity of 86.5 % and a specificity of 100 %. (125) The disadvantage of PCR based techniques are the availability, the possibility of DNA contamination and the expense involved.

HISTOPATHOLOGY

Histopathology was studied in 14 eschar biopsies of patients from Lao by Paris et al. The eschar showed necrosis of the epidermis and the adjacent epidermis showed mild spongiosis with focal inflammation. Focal parakeratosis was observed reflecting the acute process. Basal vacuolar changes and necrotic keratinocytes, focal red blood cell extravasation, intra-epidermal splitting and subepidermal blistering was probably due to necrosis. The dermis showed mild edema,

prominent mononuclear cells and macrophages and in the perivascular, interstitial, perifollicular, periglandular and focally in perineural areas. Superficial panniculitis was noted. (16)

Another study by Kim at al from Korea demonstrated similar features in 22 specimens. Dermal vascular dilatation and focal vasculitis of small vessels with lymphocytes was seen. Thrombosis of vessel wall has been noted in some instances at the base of the ulcer. Ulcerated lesions showed heavy infiltration of neutrophils in and around the small blood vessels. Lobular or septal panniculitis with predominant mononuclear cell infiltrates has also been identified. (123) Eschars have a high load of the rickettsial organism as confirmed by PCR and immunohistochemistry (IHC) studies.

Allen et al in one of the earliest studies on histopathology of eschar had studied 11 specimens. The early stage of a vesicle is seen histopathologically as an intra-epidermal vesicle which can have serum, leucocytes, mononuclear cells, blood, keratin and bacterial colonies if secondarily infected. Necrosis was seen upto the mid-dermis and was observed to reach the panniculus. Suppuration was found to be zonal though not sharply defined. Polymorphonuclear cells were found only in the upper layers. Beyond this perivascular and periappendageal mononuclear infiltrate were seen. Degenerative changes in the collagen fibres at the base of the eschar were noted as observed in spider bites. Mononuclear cells included lymphocytes, macrophages, plasma cells and mast cells. Few binucleate histiocytes as observed in tick bites were observed. Eosinophilic reaction as noted characteristically in tick bites was found to be absent. One specimen also demonstrated a part of the mite along with foreign body giant cells. Vasculitis, arteritis, non-occlusive thrombus, sub-endothelial vacuolization were noted in the vessels.

Giemsa stain also demonstrated the rickettsial organism within the endothelium of the vessel in one case. (70)

Allen et al also studied 9 specimens of the rash which he described as the 'macule' of scrub typhus. He observed an unremarkable epidermis. Edema of the papillary dermis was seen along with hyperemia of the upper dermis. Mononuclear cells were seen to eccentrically surround the vessels that included arterioles, capillaries and veins. These cells included lymphocytes, macrophages, plasma cells and mast cells. Leukocytoclastic vasculitis was observed in one specimen. Changes in the vessels as in the eschar with platelet thrombi, sub-endothelial vacuolization were present. Vasculitis of the underlying fascia and muscles were also seen. Non specific findings such as degenerative changes of the coils of the sweat glands were also seen. (70)

Classically, both the eschar and the rash in scrub typhus, show lymphocytic vasculitis. However there are reports of leucocytoclastic vasculitis reported within the eschar (16) and in some cases of the papular rash which could progress to a mixed neutrophilic -lymphocytic vasculitis. (126)

THE ROLE OF IMMUNOHISTOCHEMISTRY IN SKIN BIOPSIES

Immunohistochemistry involves the use of a specific antibody against *O. tsutsugamushi* whereby the location of the rickettsiae is identified in tissue specimens. They help in elucidating the pathogenesis of scrub typhus.

Immunohistochemical studies have been performed on autopsy specimens of 3 patients archived in U.S.A suspected to have died due to scrub typhus in World War II and the Vietnam war in an

effort to identify target cells (127) and subsequently in 2 autopsy specimens from Taiwan. (128) It was found to be located in endothelial cells of many organs and within macrophages in the lymph nodes, liver and spleen. Acute tubular necrosis in a patient was identified to be due to direct invasion by *O. tsutsugamushi* using IHC methods. (94) A recent case report from Korea demonstrated the use of IHC in liver biopsy specimen to identify scrub typhus infection in a patient with fever and hepatitis. (71)

IHC of lesional skin biopsies have been developed as diagnostic methods for spotted fever (129) and rickettsial pox. (130) IHC studies have been performed for skin lesions of scrub typhus patients from Korea with all 22 specimens of eschars showing 100 % positivity with the Boryong strain. IHC studies of scrub typhus eschars have shown that the organism mainly infects antigen presenting cells especially around the necrotic zone as well as in monocytes and macrophages. Neutrophils were found at the delineating zone of necrosis.

Presence of the organism within endothelial cells was seen rarely in a recent study as against previous studies .*O. tsutsugamushi* was found mainly within antigen presenting cells like monocytes, macrophages and dermal dendritic cells. The *O. tsutsugamushi* organisms within these cells were intact and formed clusters often near the nucleus implicating intracellular replication. B-cells were very rarely infected and T-cells often showed the organism on its surface than intracellularly. (16)

The yield of detecting the organism with IHC in the maculopapular rash (n=24) was lesser. It had a sensitivity of 65% only while the specificity was 100%. (123)

TREATMENT

The arrival of chloramphenicol in 1947 was a milestone in the treatment of scrub typhus. Research led to the use of terramycin (oxytetracycline hydrochloride), aureomycin (chlortetracycline hydrochloride) and para-aminobenzoic acid tetracycline in this disease. (131) Tetracycline was found to be more effective than chloramphenicol both of which are not used in the present scenario. Both tetracycline and chloramphenicol are avoided in pregnancy and childhood. Intravenous minocycline has similar effects. Macrolides such as azithromycin and roxithromycin are also being used. Azithromycin has been found to be as effective as doxycycline and has better gastrointestinal tolerability. Doxycyline showed faster defervescence than roxithromycin in one trial. Rifampicin may be an option in doxycycline resistant areas. Meta-analysis of treatment efficacy has shown that doxycycline is the preferred option all cases of mild infection. The regime consists of doxycycline 100 mg twice daily for 7 to 14 days. (132)

Defervescence of fever within 24 to 48 hours with appropriate antibiotics is a characteristic feature so much so that it can raise the index of suspicion of a rickettsial infection in a patient with fever of unknown origin. However there are reports of delayed defervescence i.e., more than 3 days with doxycycline as reported from China by Lai et al (133) raising concerns of resistance to doxycycline. Studies from Thailand have shown doxycyline and chloramphenicol resistance. (134)

MEASURES TO CONTROL DISEASE

Control of the vector has been suggested by the use of insecticides like dieldrin, aldrin, chlordane, toxaphene, lindane, Malathion, the use of insect repellants like N, N-diethyl-m-toluamide (DEET) to prevent chigger bites and the treatment of clothing with DEET, permethrin. The use of protective clothing with long sleeves, long pants and socks has been suggested. (135)

Elimination of hosts like rodents has actually been found to increase the presence of unattached mites. The effect of burning of vegetation lasts only till the soil dries out. Systemic insecticide as baits for rodents was not effective for immediate control. The most obvious way to control this disease is to prevent humans from going to mite infested areas which may not always be feasible.

Chemoprophylaxis with chloramphenicol and doxycycline was studied in large populations of military personnel though not recommended at present.

An effective vaccine has not yet been developed due to difficulty in producing antigens since

O. tsutsugamushi grows slowly in cultures, has varied strains and due to its poor immunogenicity.

MATERIALS AND METHODS

STUDY DESIGN

Observational, cross-sectional study

SETTING

***Location*:** Our institution is a tertiary care hospital that caters to about 3000 in-patients with fully equipped emergency medicine services set in the endemic area of Vellore. Vellore has tropical dry and hot climatic conditions for most part of the year with a maximum of 40.5 degree Celsius in April-June and a minimum of 18.4 degree Celsius in November-January. The cooler months of the year are frequented by rainfall. Maximum rainfall occurs with the north east monsoons during September to October. Rainfall also occurs from June to September with the south west monsoons. (4) Studies have shown that scrub typhus occurs during the cooler season of the year in Vellore. We conducted this study by recruiting patients from the Emergency Medicine, wards of General Medicine, Medical Intensive Care Unit (MICU) and Medical High Dependency Unit (MHDU) at Christian Medical College Hospital, Vellore

***Period of recruitment*:** 13 months (October 2011 till October 2012)

***Exposure*:** Patients with scrub typhus

Follow up: Patients were observed during their hospital stay to assess response to treatment and complications.

Data collection: At entry (admission to emergency medicine, MICU and MHDU) and during hospital stay

PARTICIPANTS

Inclusion criteria:

All adult patients (>15 years of age) with a febrile illness of 5 - 28 days duration with a

(1) Positive serum IgM ELISA for scrub typhus, **or**

(2) The presence of an eschar

Exclusion Criteria

(1) other proven systemic infections like Dengue, Leptospirosis, bacterial infections

(2) positive spotted fever IgM ELISA in patients presenting only with acute febrile illness and rash

(3) patients with serious accompanying systemic diseases like malignancies and autoimmune disorders

(4) patients unwilling to participate in the study

Withdrawal criteria

Patients unwilling to continue participation in the study

METHODOLOGY

Step 1: Sample size calculation

Based on previous studies the prevalence of skin lesions was taken as 50 %. Formula for estimating single proportion (Absolute precision) = $(Z_{1-\alpha/2})^2 \times p\ (1-p)/\ d^2$

Where,

p = Expected proportion was taken as 50% i.e., 0.5

d = Absolute precision was taken as 10 % i.e., 0.1

1-α/2 = Desired Confidence level

Sample size = $1.96^2 \times 0.5 \times 0.5 / (0.1)^2 = 96$

The sample size was calculated to be 96 patients.

Step 2: Identification and recruitment of patients

Patients admitted to the Emergency Medicine, Medical wards, MICU and MHDU with acute febrile illness i.e., fever from 5 to 28 days duration were screened for skin lesions, specifically eschar and / or a rash by the primary investigator. Patients with a positive eschar or with a positive scrub typhus IgM ELISA (InBios International, Inc. USA), which was a qualitative serological test (done as a diagnostic test as part of evaluation of the acute febrile illness by the physician), were recruited into the study after obtaining informed consent (Appendix IV). In patients with acute febrile illness and rash without eschar, spotted fever was to be excluded by spotted fever ELISA. Patients also underwent PCR of the scab of the eschar, when present. Patient recruitment was not possible when patient death occurred within the first 24 hours.

Step 3: Data collection

Data was recorded in data collection forms (Appendix II). It included:

1. Demographics – age, sex, hospital number, occupation, place
2. Presence or absence of eschar was noted
3. The location of the eschar was represented on a body chart
4. The size horizontal and vertical (diameter of the eschar) was measured using an plastic ruler
5. Rash or any other skin lesions if present was documented
6. Clinical photographs of skin lesions were taken
7. Duration of fever at admission was documented
8. Laboratory investigations that were noted included white blood cell counts, platelet counts, serum creatinine, hepatic function tests like bilirubin levels, SGOT/AST, SGPT/ALT and results of IgM ELISA for scrub typhus if available were documented
9. The antibiotic used for treatment was recorded and defervescence of fever was noted during hospital stay
10. Duration of hospital stay was recorded
11. Any complications were noted like aseptic meningitis, acute respiratory distress syndrome

Step 4: Biopsy of the eschar or rash

The edge of the eschar or ulcer (if the necrotic scab was absent) or the rash was biopsied after obtaining consent. A 4 mm sterile disposable skin biopsy punch manufactured by Cardiograph

Corporation, Mumbai was used. Cotton thread was used to suture the biopsy site.
The biopsied tissue was placed in 10 % formalin for fixation and paraffin embedded in the

pathology laboratory. Their histology was studied using haematoxylin and eosin stain.

Step 5: Analysis: Descriptive statistical methods were used to summarize the demographic and other study variables. A database was created in EpiInfo v.7 and subsequently analysed using SPSS software. Statistical analytic methods like chi-square test, fisher's test, t- test, Mann-Whitney U test and Kruskal Wallis test were used in the analysis of data.

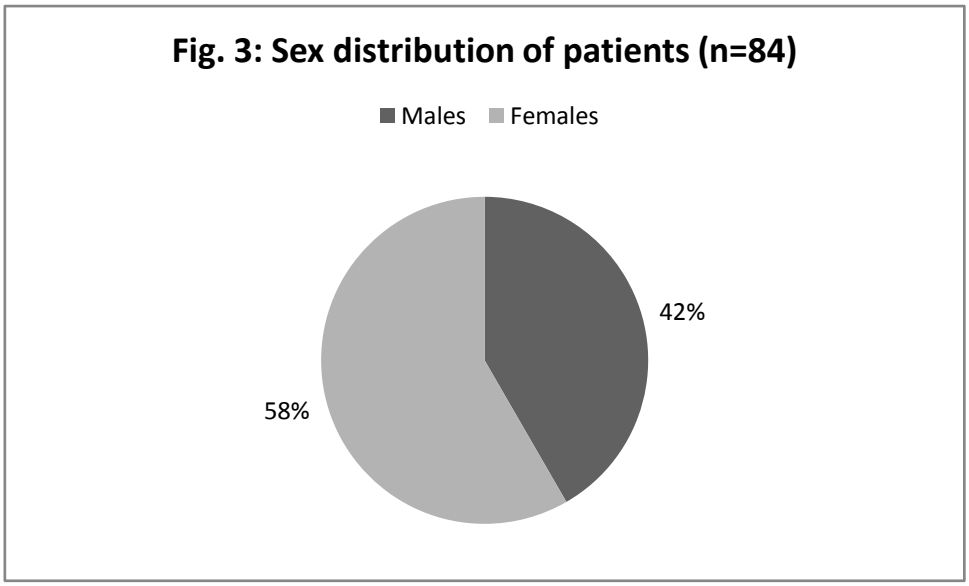
INSTITUTIONAL RESEARCH BOARD APPROVAL AND ETHICAL CONSIDERATIONS

Institutional Research Board (IRB) and ethical committee approval was obtained prior to commencement of the study. Written consent was obtained from the patient or next of kin for inclusion into this study

RESULTS

DEMOGRAPHICS

We conducted a study to describe the cutaneous manifestations of scrub typhus which is a zoonotic infection transmitted by the *leptotrombidium* mite. We looked at patients coming to our hospital, a tertiary care hospital set in Vellore, Tamil Nadu known to be an endemic area. The study period was from October 2011 to October 2012. There were 352 scrub typhus patients in this period of which 84 patients were recruited into the study. Of these, 70 patients were hospitalized and the rest of the 14 were patients who were discharged from emergency medicine following a short period of observation. There were slightly more number of females than males. Among 84 patients, 35 cases (41.6%) were males and 49 cases (58.3 %) were females **(Fig. 3).**



PLACE

The endemicity of the disease was studied. We found that patients in our study hailed from Vellore, Tiruvannamalai, Krishnagiri, Chittoor and Cuddapah **(Fig. 4)**. The geographical location is marked in the map **(Fig. 5)** which shows a clustering of cases in and around Vellore upto 200 km away. Most patients were from Vellore, followed by Chittoor which is in the neighbouring state of Andhra Pradesh.

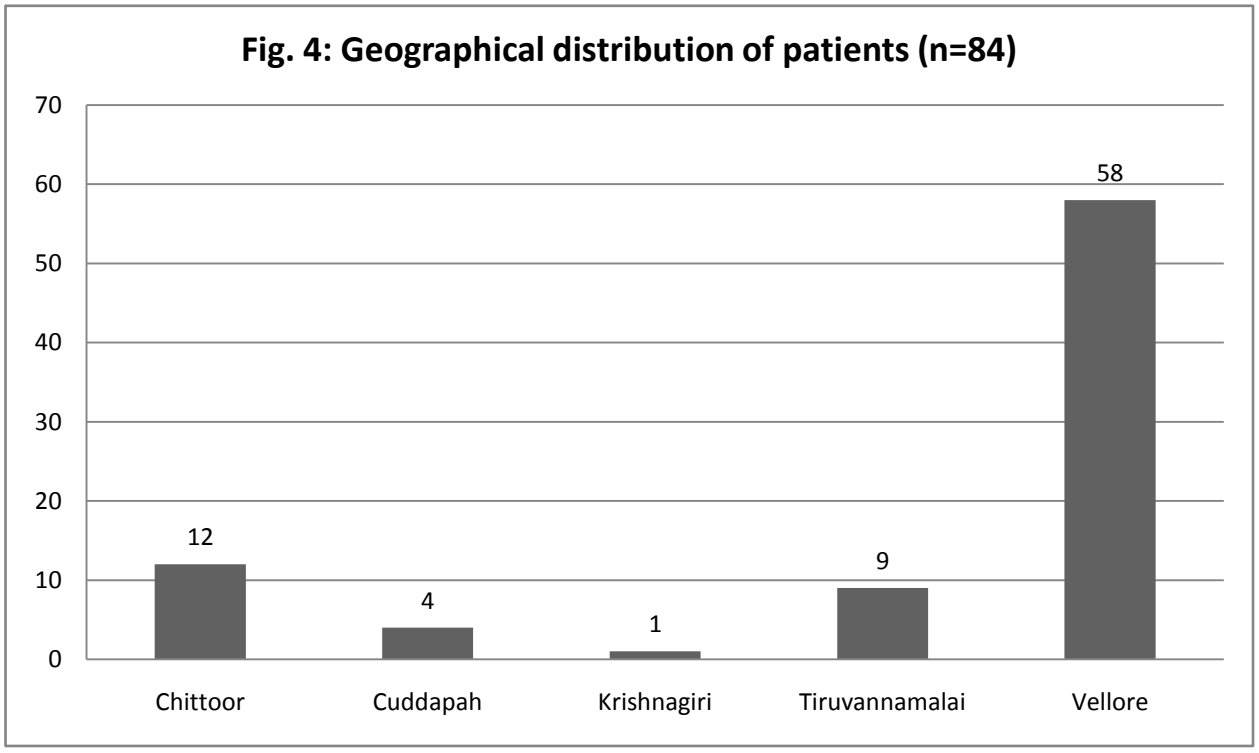


Fig. 5a: Distribution of all study cases of scrub typhus

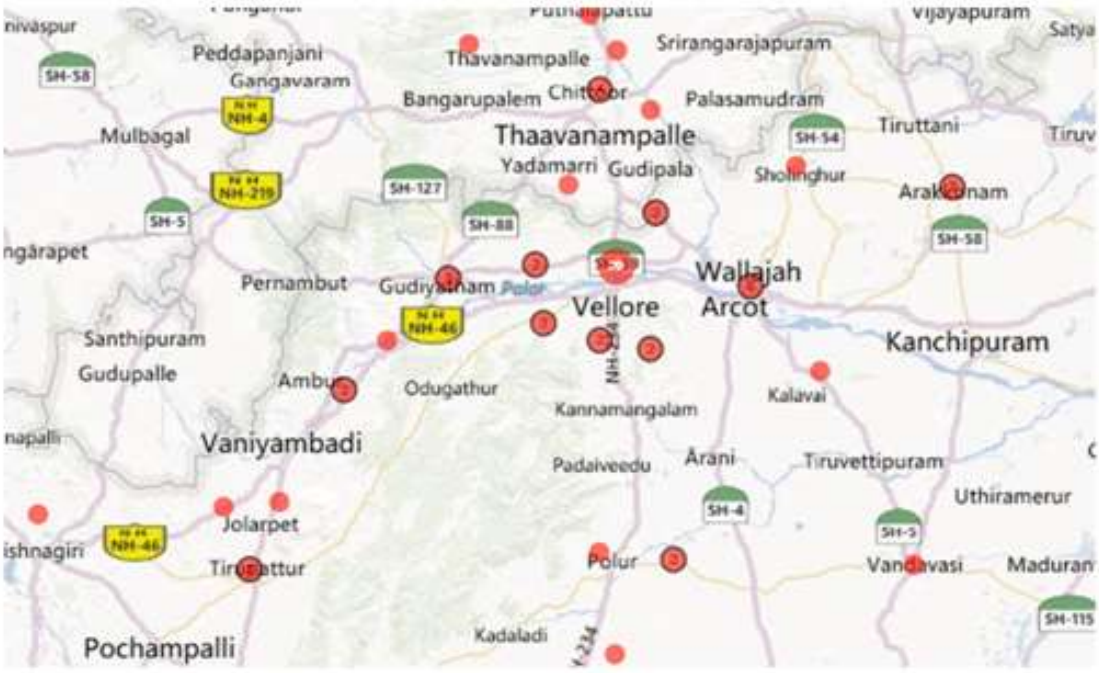
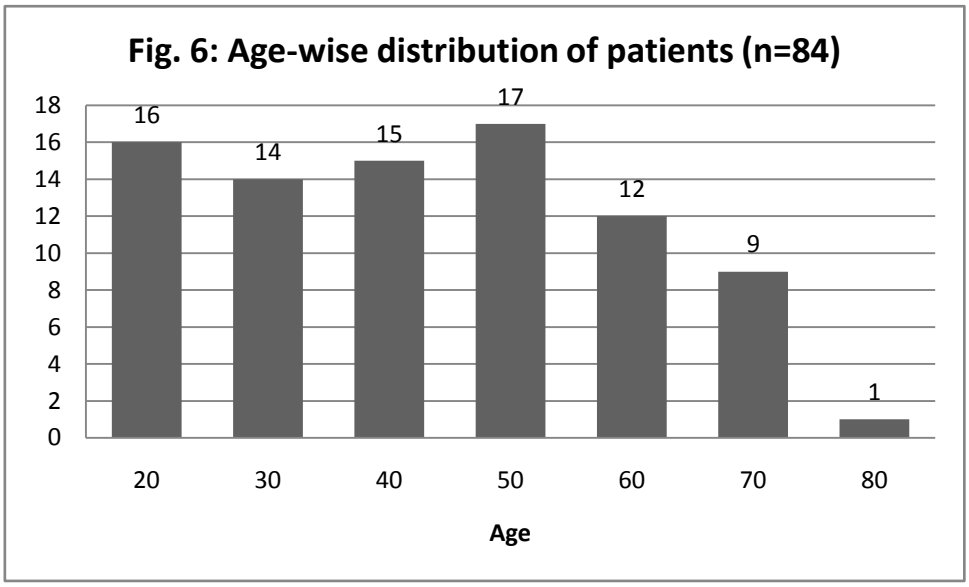


Fig. 5b: Distribution of study cases of scrub typhus in Vellore

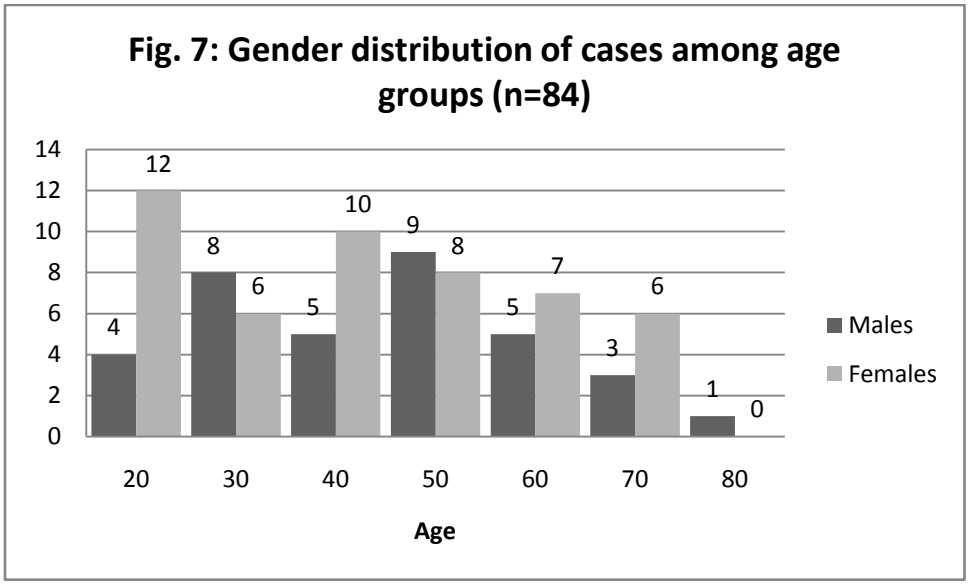
Fig. 5 Geographical distribution of cases *(plotted on Epi-info v.7)*

AGE

Our study included only adult patients. The age of patients ranged from as young as 18 years to as old as 80 years, with a mean age of 44.4 ±17 years. The age distribution showed a maximum peak in the 45-55 year age group with a similar distribution in younger age groups (15-25, 25-35 and 35-45 year age groups), all totally accounting for 73.8% of patients (n=84). **(Fig. 6)**



The gender-wise distribution of cases among various age groups was analysed. The mean age of females was 43.3±17 years and the average age of males was 45.9±17years. There was no statistical difference in the age-distribution of cases according to gender (independent samples test, p value=0.48) Maximum number of females were in the age group of 15-25 years whereas more number of males belonged to the age group of 45-55 years; but this was not significant (Fisher's exact test, p value = .206). **(Fig. 7)**

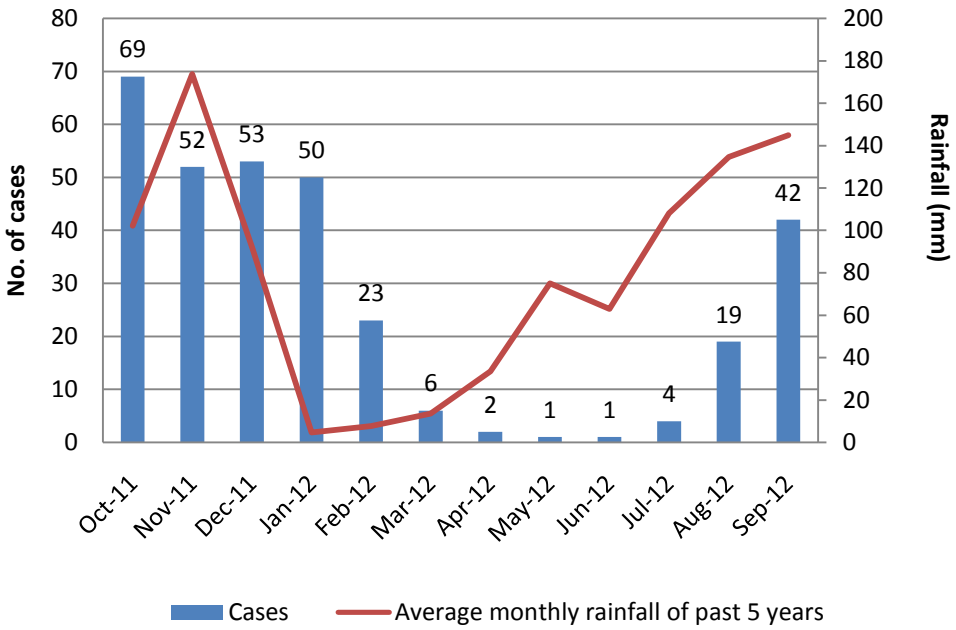


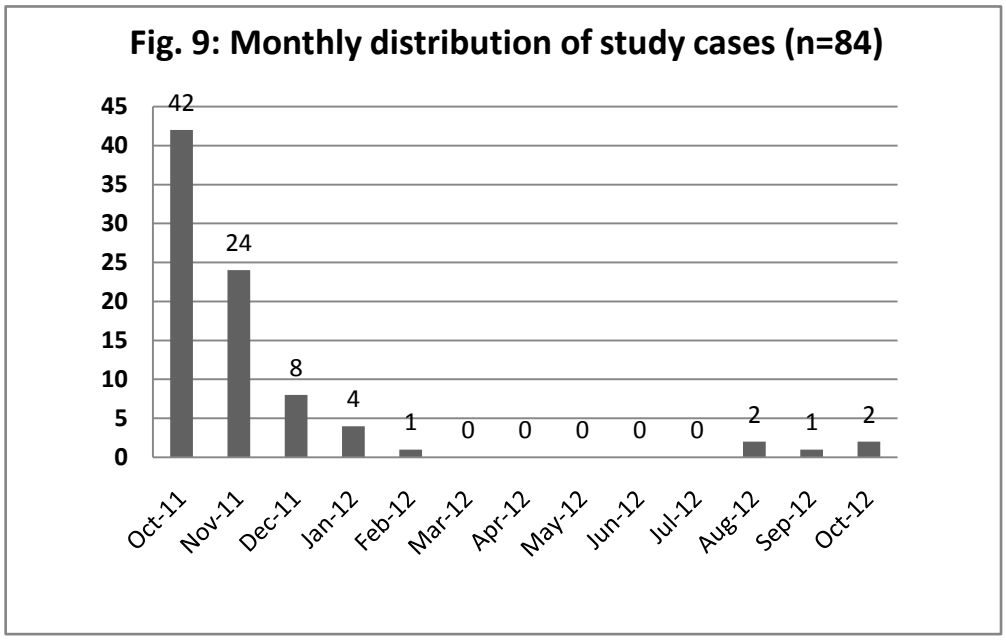
MONTHLY DISTRIBUTION

The study period was from October 2011 till October 2012. In this period it was found that the maximum number of cases was reported in the month of October in the year 2011. The average of monthly distribution of rainfall for the past 5 years in the district of Vellore was obtained from data of the Indian Meteorological Department (Ref: http://www.imd.gov.in/section/hydro/distrainfall/webrain/tamilnadu/vellore.txt) and plotted against the monthly distribution of total cases (n=352) that had presented to our hospital through 13 months **(Table 1).** It was found that maximum numbers of cases occurred immediately following rainfalls and subsequently reduced when the rainfall ceased **(Fig. 8**). Only 10 cases were reported from March 2012 till July 2012. The distribution of study cases followed a similar pattern **(Fig. 9)**.

Table 1: MONTHLY DISTRIBUTION OF CASES AND AVERAGE RAINFALL		
	Total no.of cases (n=352)	**Average rainfall in Vellore (mm)**
Oct-11	69	102.12
Nov-11	52	173.68
Dec-11	53	92.02
Jan-12	50	4.66
Feb-12	23	7.66
Mar-12	6	13.62
Apr-12	2	33.48
May-12	1	74.96
Jun-12	1	62.88
Jul-12	4	108
Aug-12	19	134.64
Sep-12	42	144.86
Oct-12	30	102.12

Fig. 8: Monthly distribution of cases (n=352)





OCCUPATION

Of all the reported cases of scrub typhus, most patients were manual labourers who engaged in agricultural activities. They formed 51.2% (43/84) of the study group. The various types of occupation included housewives, house maids, drivers, salesman, office staff, nurse, student, unemployed and retired men. Except for one patient, all reported direct contact with fields and vegetation. The patient who did not have any exposure to vegetation was a salesman by occupation. **(Table 2)**

Table 2: DISTRIBUTION OF OCCUPATIONS OF CASES AMONG MALES AND FEMALES (n=84)		
Occupation	**Males (n=35)**	**Females (n=49)**
Manual/agricultural labourer	23	20
Housewife	-	24
Retired/unemployed	4	0
Office staff	2	1
Driver	2	0
Nurse	0	2
Salesman	2	0
Student	2	0
Misc	0	2

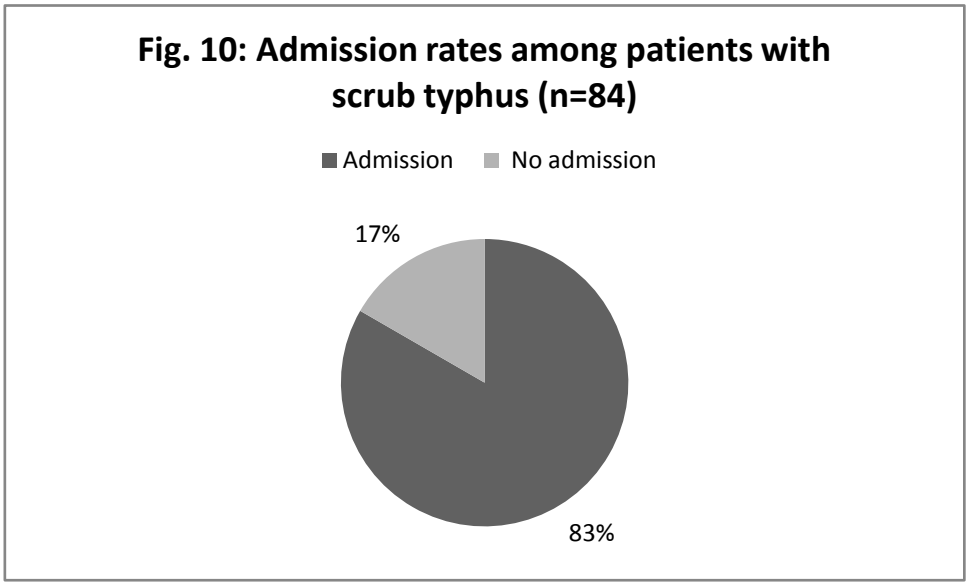
DURATION OF FEVER AT HOSPITALISATION

Patients on presenting to the hospital had an average duration of fever of 9.9±4.4 days (range of 5-30days). We analysed the differences in the mean duration of fever at hospitalization in groups of patients with and without complications arising from scrub typhus and found no statistical difference between the two (Student's two tailed t-test, p value – 0.9260).

HOSPITAL STAY

Of the 84 cases, 70 patients required hospitalization and 14 were discharged on medications after a diagnosis was made in the Emergency Department **(Fig. 10)**. The average duration of stay in hospitalized patients was 7.17±4.4 days. The maximum duration of hopitalisation was 25 days.

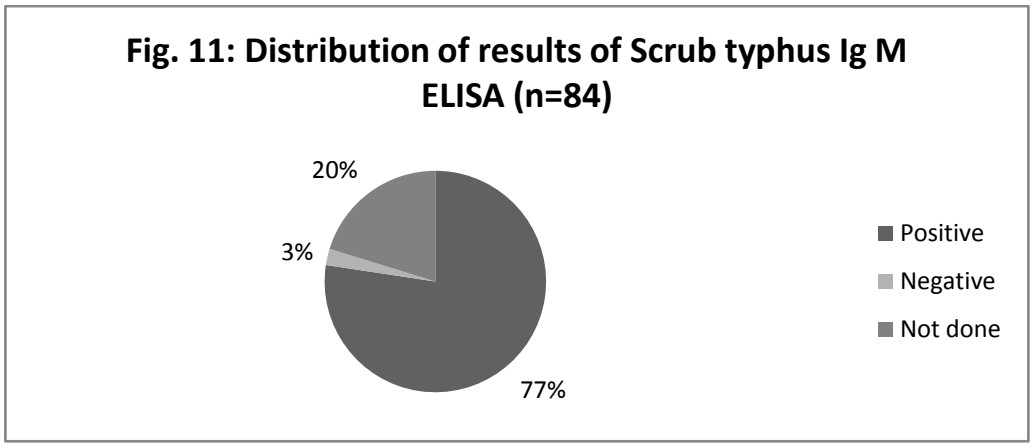
Fever defervescence was assessed only in 67 patients and the mean time taken for the fever to defervesce was 1.9±0.98 days. 4/67 (6%) patients took longer than 3 days for fever to subside; 2 patients took 4 days while the other 2 took 5 days. The common antibiotics used were doxycycline and azithromycin.



SEROLOGY

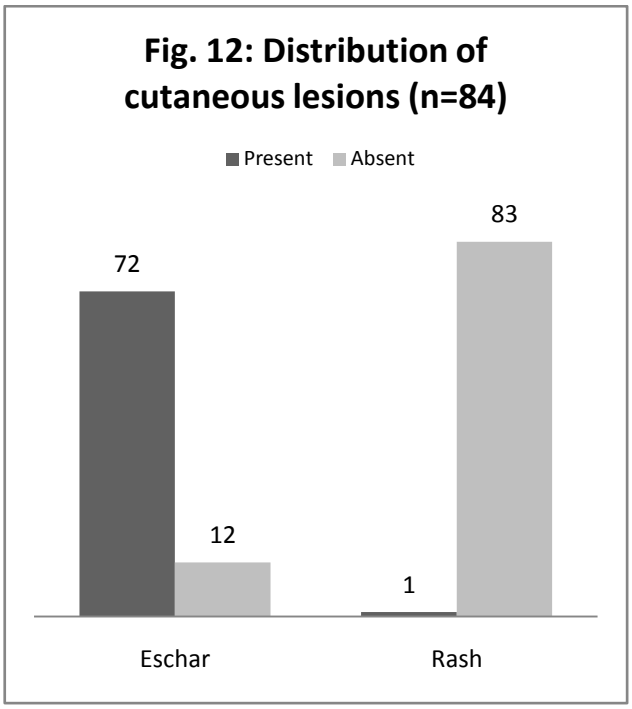
Of 84 patients, 67 patients had serology done for scrub typhus IgM ELISA of which 2 were negative **(Fig. 11)**. Of the negative patients, both had eschars though one patient had grouped eschars which on biopsy showed a lymphocytic vasculitis and the other patient had evidence of a lymphocytic vasculopathic reaction. Both patients responded to doxycycline. One patient was tested after 7 days of fever, on the 3rd day of hospitalization and the other patient was tested after 5 days of fever.

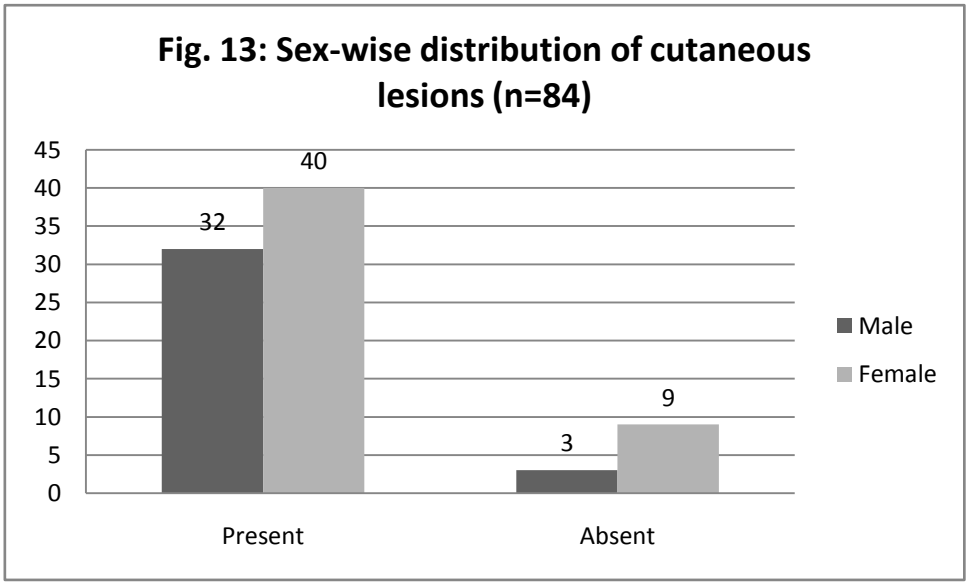
12 patients did not have cutaneous lesions of scrub typhus but had positive scrub typhus IgM ELISA. Of these one patient had negative serology for both dengue and leptospirosis whereas 2 were tested negative for dengue alone and 3 others were tested negative for leptospirosis alone. Typical fever defervescence with doxycycline and azithromycin (i.e., defervescence within 48 to 72 hours) was noted in 11 of these patients. Data of fever defervescence was missing in one case.



CUTANEOUS MANIFESTATIONS

All patients were examined thoroughly for cutaneous lesions. Most (72/84) patients had cutaneous manifestations (85.7%). All the patients with cutaneous manifestations had eschars and one had a concomitant rash also. 12/84 patients did not have any lesion on the skin even after a thorough search. **(Fig. 12)** Of those with eschars, there were 32 males and 40 female patients. Of those without eschars, there were 9 females and 3 males. **(Fig. 13)**

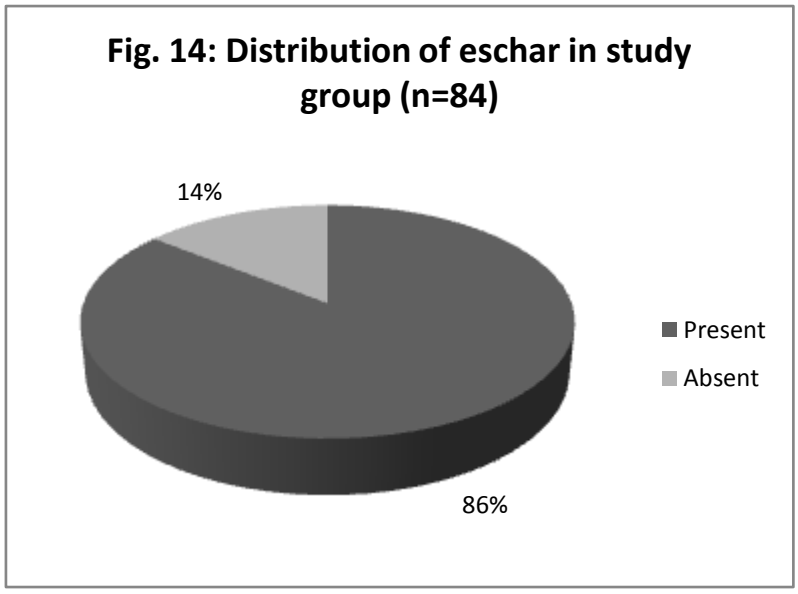




ESCHAR

72 patients in the study group presented with eschars; one of whom had a concomitant rash. They were widely distributed over different regions of the body **(Fig. 14).** Not all patients in our study had the typical necrotic scab of an eschar. 21/72 patients were found to have well defined

ulcers that appeared punched out with a rim of erythema. Maximum number (7/21) was located over the inner aspect of the thigh.



Distribution of eschars by body site

The trunk was found to have eschars more commonly (33/72, 45.8%). Of these 1 was infraclavicular, 4 were on the breasts, 2 over the inframammary area, 4 over the chest wall, 7 over the upper abdominal wal , 6 over the lower abdominal wall , 4 over the scapular region and 5 over the lumbosacral areas. The next commonest site was the lower limbs (11/72, 15.3%). The flexural regions like the axilla (9/72), groin (6/72), genitalia (5/72), neck (4/72) and upper-limbs (4/72) were less frequently involved.

63/72 (87.5%) eschars were present over the anterior aspect of the body and 9 (12.5%) were present over the posterior aspect of the body. 30 males had the eschars on the anterior aspect and 2 had on the posterior aspect of the body. 33 females had eschars on the anterior aspect and 7 had

them on the posterior aspect of the body. However, there was no statistically significant difference in the distribution between genders (chi-square test, p value=0.282).

21/72 presented as discrete ulcers without the overlying necrotic scab. These were found mainly in the inner aspect of the thigh (7/21), axilla(3/21), scrotum (2/21), breast (2/21), chest wall (2/21), 1 each over the groin, perineal area, inner aspect of the upper arm and the lumbar area.**(Fig.15,16,17)**

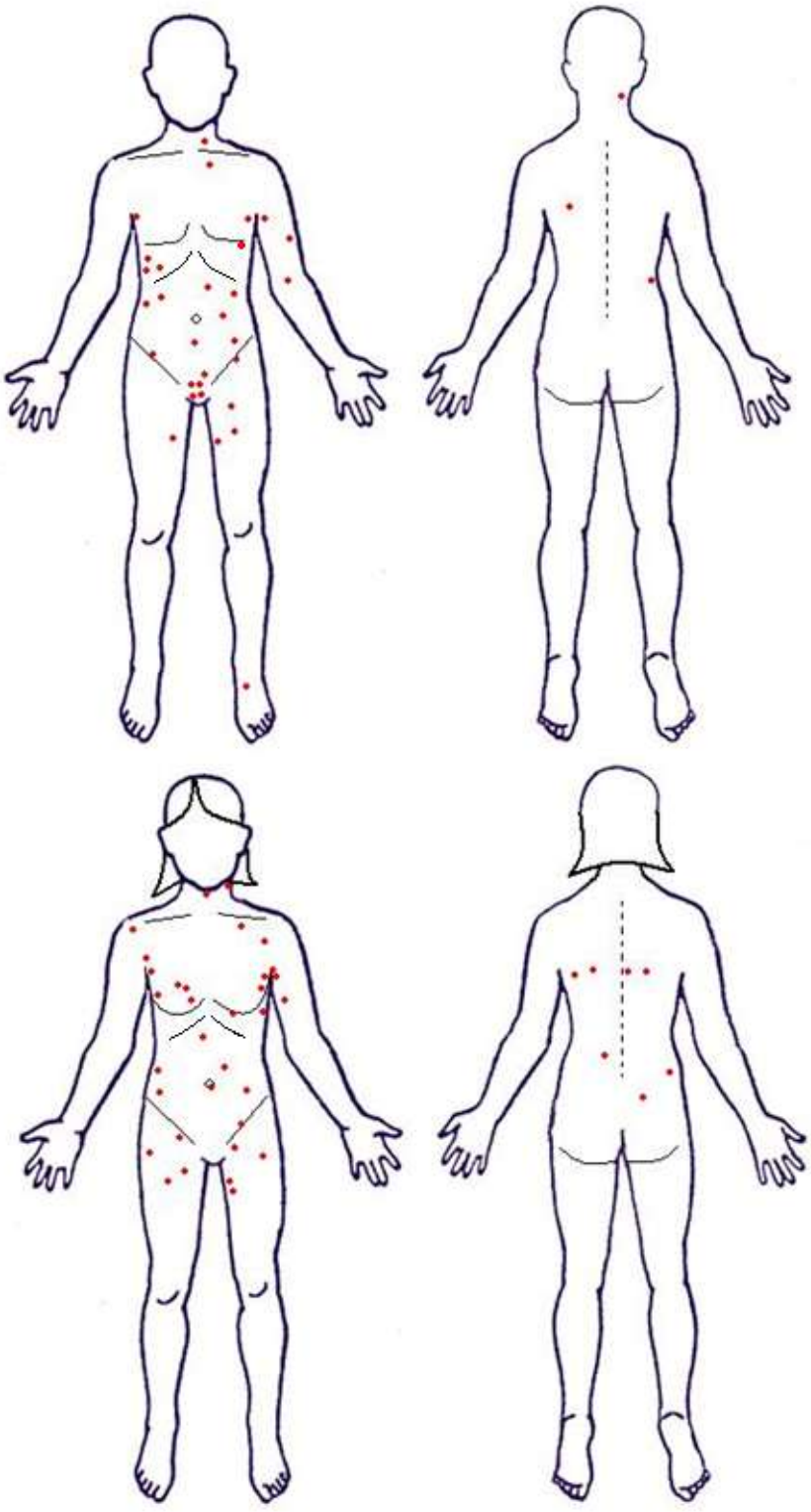


Fig. 15: Pictorial representation of eschar distribution

Neck

Nape of neck

Shoulder

Infraclavicular

Breast

Axilla

Fig. 16a: Eschars at various sites

Umbilicus

Cubital fossa

Trunk

Groin

Waist

Lower abdomen

Fig. 16b: Eschars at various sites

Groin

Thigh—inner aspect

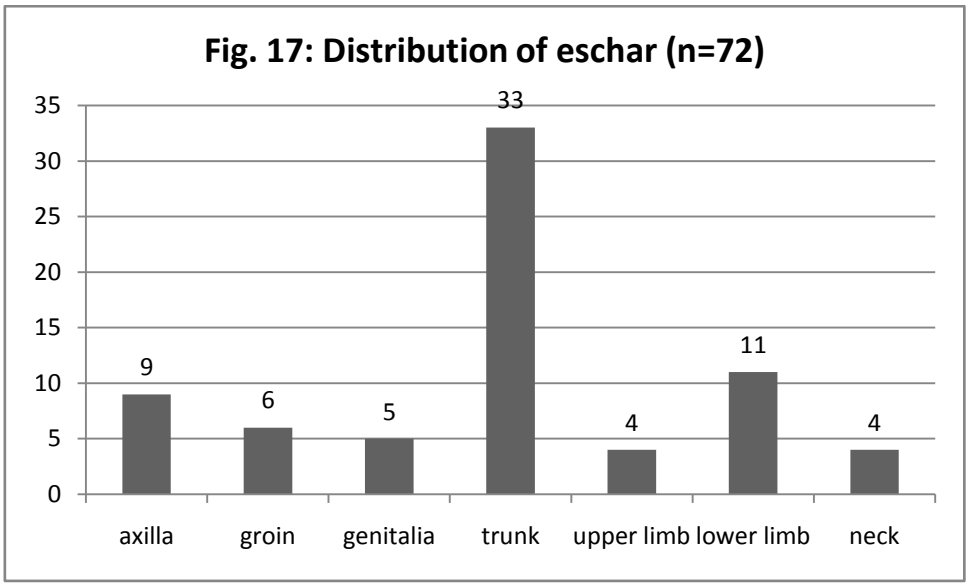
Thigh—outer aspect

Scrotum

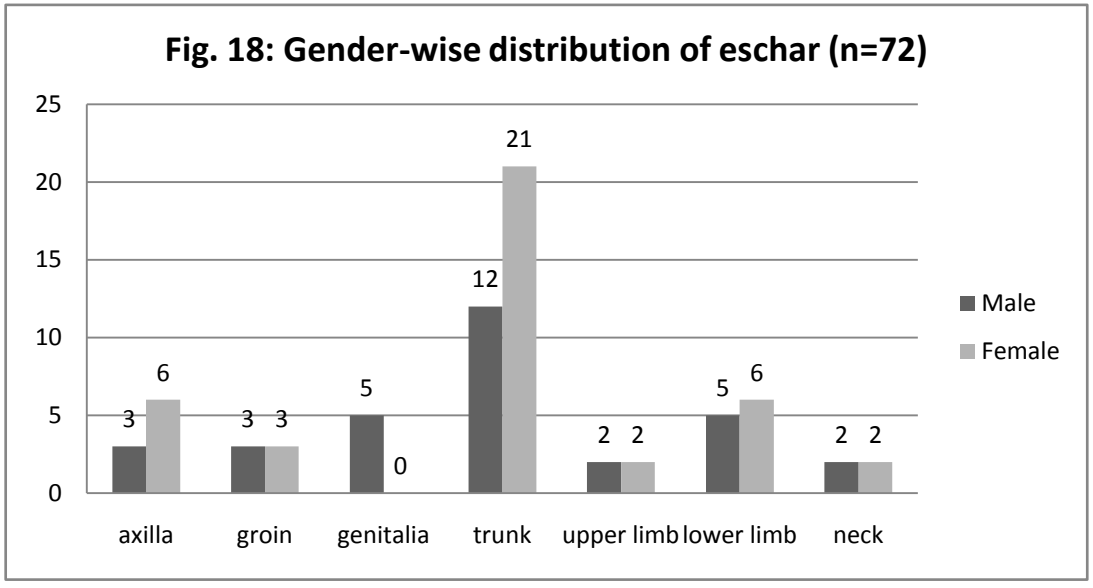
Scrotum

Rash

Fig. 16c: Eschars at various sites and rash



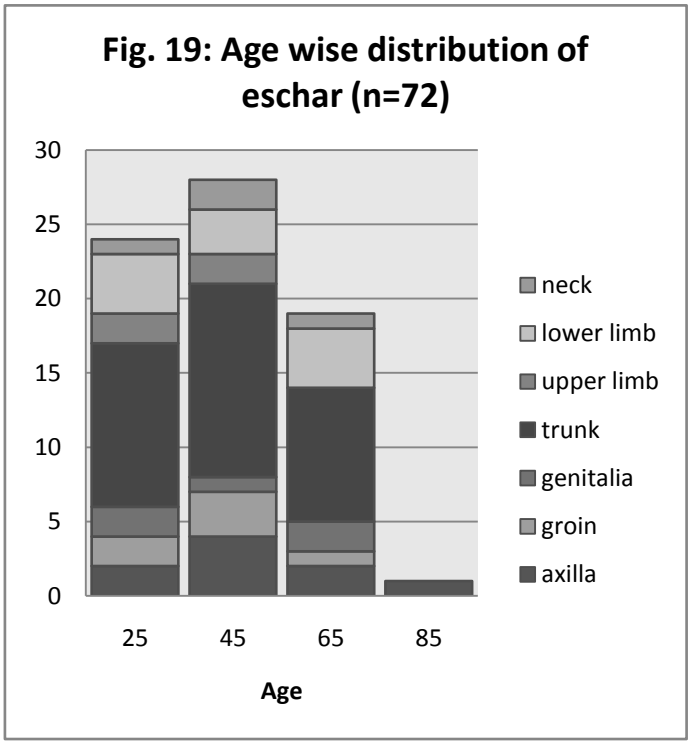
The distribution of eschars among males and females was analysed and it was found that eschars over the trunk were found predominantly in females (21/33) while genital lesions were found exclusively in males **(Fig.18).** There was, however, no significant difference between both groups (fisher's exact test, p value = 0.235). We also examined the distribution when categories were combined as flexures (axilla and groin) and limbs (upper and lower limbs) and found no significant difference (Fisher's exact test, p value=0.150)



We analysed the age wise distribution of the eschar. There was no significant difference between the distributions of eschar **(Table 3, Fig. 19).**

Table 3: DISTRIBUTION OF ESCHARS ACCORDING TO VARIOUS AGE GROUPS

Age group	Axilla	Groin	Genitalia	Trunk	Upper limb	Lower limb	Neck
15-35	2	2	2	11	2	4	1
36-55	4	3	1	13	2	3	2
56-75	2	1	2	9	0	4	1
76-95	1	0	0	0	0	0	0



Number of eschars

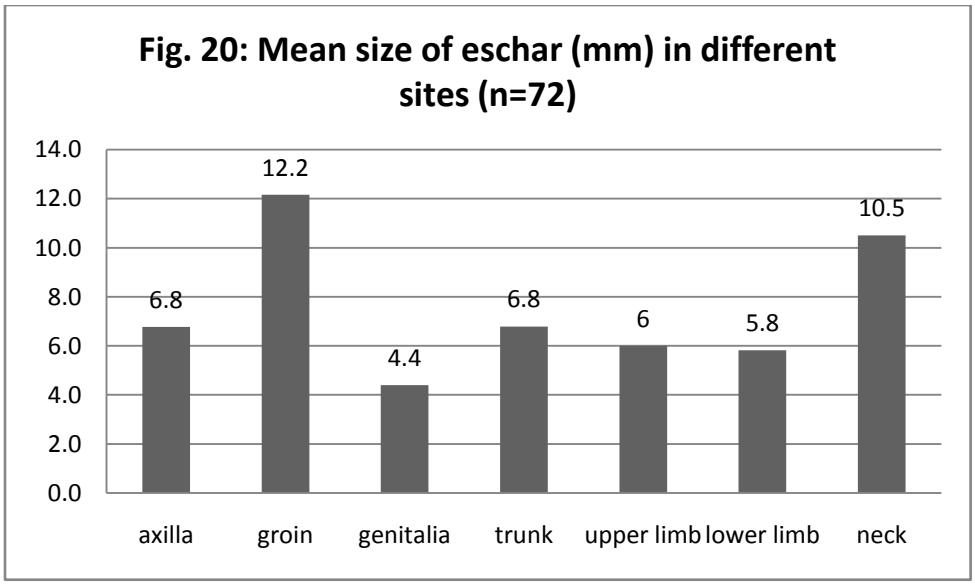
70/72 patients (97.2%) had a single eschar. Multiple eschars were seen in 2/72 (2.7%) patients. One of them had 2 eschars and another had 3 grouped eschars. Of these one patient had serum IgM ELISA positive for scrub typhus and the other patient had typical fever defervescence with doxycycline.

Size

The eschar size was recorded in two perpendicular diameters i.e., horizontal and vertical and the largest value in diameter was taken as eschar size. The mean eschar size was 7.1±4.5mm. The smallest eschar noted was 3x2 mm in 3 patients while the largest was of size 25x9 mm.

Females were found to have larger eschars as compared to males with a mean of 7.5±4.5 mm in females and 6.5±3.3 mm in males. However this was not statistically significant (Mann-Whitney U test, p value = 0.945)

The mean size of eschars at different sites of the body was also looked at. The largest eschars were found in the groin and on the neck with a mean greater than 10mm **(Fig. 20)**. We analysed this using the Kruskal Wallis test and found this to be statistically significant with a p value of 0.048 (<0.05).



It was found that 15/72 eschars (20.8%) were less than 5 mm. Scrub typhus IgM ELISA was done in 14 of these patients and 13 of them were positive while one was negative for the serological test.

Absence of eschar

Eschar was absent in 12 patients (14.3%). Of those with absent eschars a larger proportion was females (10/12). However, this was not a significant finding (Fisher's exact test, p value = 0.224).

We analysed the various clinical parameters to see if there was any adverse outcome with absence of eschars. We had looked at duration of fever and hospitalization and various laboratory parameters like total WBC counts, platelet count, SGOT, SGPT and creatinine levels.

Any organ failure, shock or significant organ involvement was considered as a complication. There was no statistical difference between various parameters as shown in the table. The Student's t-test was used to analyse the means, Mann-Whitney U test to analyse the duration of fever and hospitalization and Fisher's test was used for the analysis of complications **(Table 4).**

MACULOPAPULAR RASH

Rash was present in only one patient who had a concomitant eschar. It was a discrete, erythematous, papular rash on the trunk and extremities sparing the palms and soles.

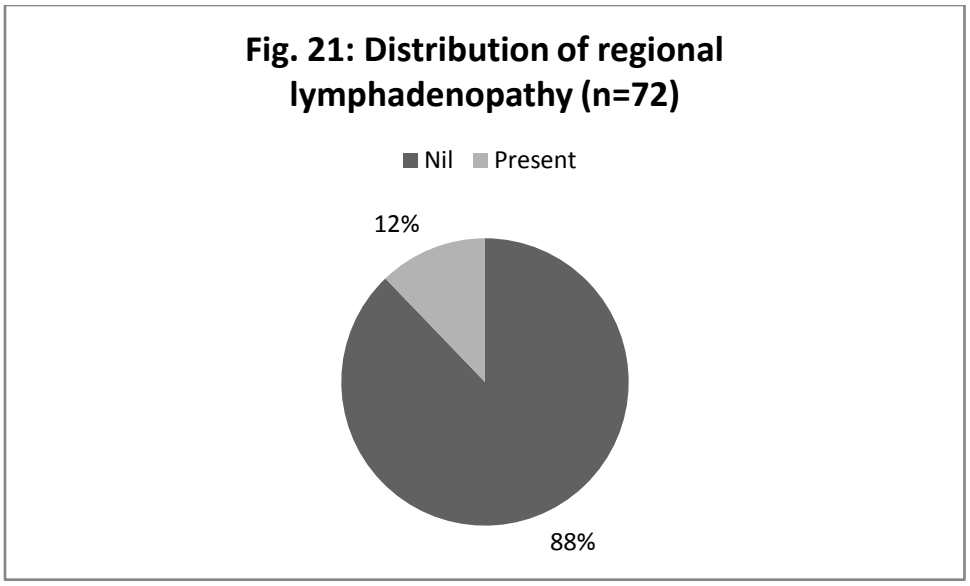
Table 4: LABORATORY PARAMETERS AND CLINICAL OUTCOME IN PATIENTS WITH AND WITHOUT ESCHAR

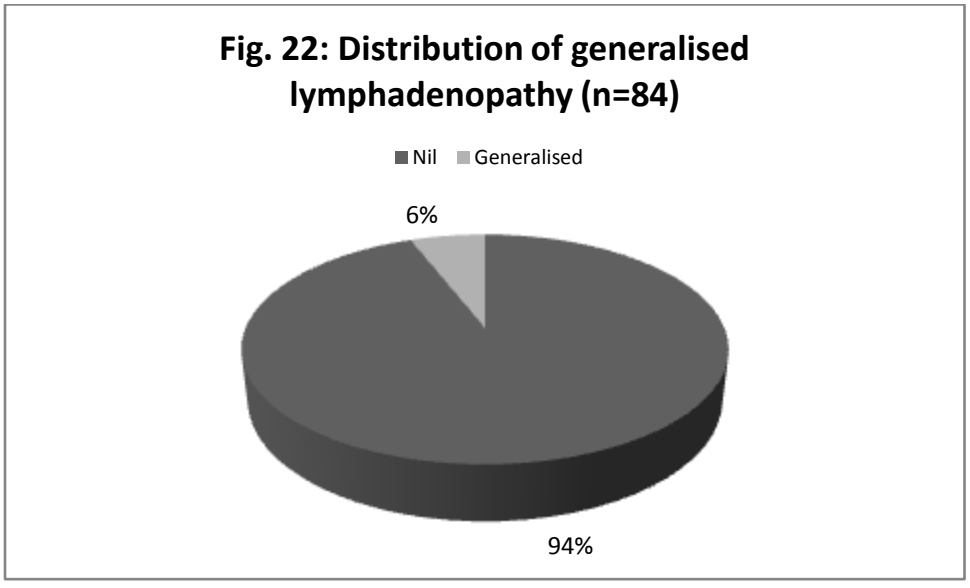
	Eschar	**No eschar**	**p value**
Duration of fever (days)	10.01	9.38	0.848
Duration of hospitalization (days)	7.3	7.18	0.393
Total WBC (per mm^3)	11652.7	10415.4	0.399
Platelet (per mm^3)	88028.2	106307.7	0.323
SGOT (U/L)	132.6	147.3	0.625
SGPT (U/L)	75.3	84.7	0.614
Creatinine (mg %)	1.49	1.36	0.691
Complication	39/72	8/13	0.590

LYMPHADENOPATHY

Regional and generalized lymphadenopathy was found only in few patients. In most patients lymphadenopathy was absent.

Regional lymph nodes were found only in 13.8 % (10/72) of patients. Generalized lymphadenopathy was found only in 6 % (5/84) **(Fig. 21, 22)**





LABORATORY PARAMETERS

Leucocytosis

The total WBC count was elevated (>11,000 /mm^3) in 38/84 patients (45.2%). The average total WBC count was 11,469±5087/mm^3. In patients with complications, the mean WBC count was 13012.8±5203/mm^3 and in those without complications it was significantly lower at 9547.4±4265 /mm^3 (p value = .001). **(Table 5)**

Transaminitis

SGOT was the most commonly elevated transaminase (79/84, 94%). SGPT was elevated in 67/84 patients (79.7%). The mean value of SGOT was 133.9±89.6 U/L and the mean value of SGPT was 76.8±57.3 U/L. There was no statistically significant difference in the values between patients with and without complications. **(Table 5)**

Platelet count

Platelet counts were abnormal (<100,000 /mm^3) in 53/84patients (63%). The mean value of platelet count was 90654.7±59166 /mm^3. There was no significant difference between groups of patients with and without complications. **(Table 5)**

Creatinine

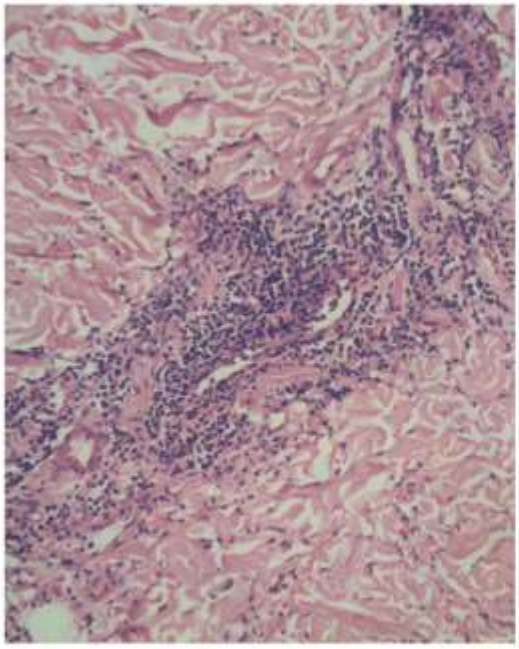
Deranged values of creatinine (>1.4 mg %) were seen in 23/84 patients (27.3%). The mean value of was 1.48±0.98 mg% and there was no statistically significant difference between values in groups of patients with and without complications. **(Table 5)**

Table 5: LABORATORY PARAMETERS IN PATIENTS WITH AND WITHOUT COMPLICATIONS			
	Complication	**No complication**	**p value**
Total WBC (per mm^3)	13012.8	9547.4	0.001
Platelet count (per mm^3)	93021.7	88236.8	0.714
Creatinine (mg %)	1.55	1.38	0.423
SGOT (U/L)	146.4	120.3	0.189
SGPT (U/L)	80.2	72.3	0.535

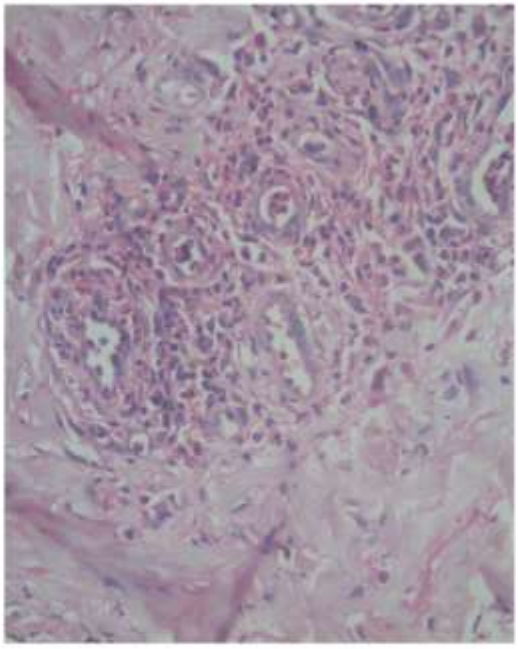
HISTOPATHOLOGY

43 patients had consented for biopsy of the skin lesion i.e., the eschar. Light microscopy with H&E stain showed features of vasculitis, panniculitis or vasculopathy.

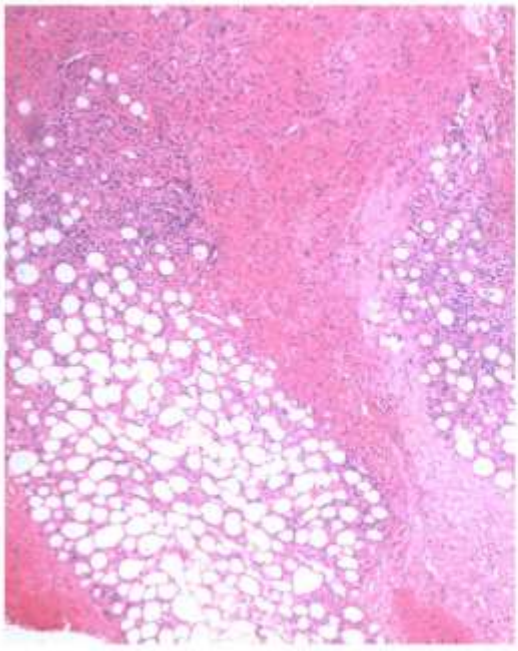
Vasculitis was present in 32/43 specimens (74.4%). Of these, 24 patients had lymphocytic vasculitis (75%), 2/32 had neutrophilic (leukocytoclastic) vasculitis (6.25%) and 6 /32 had mixed vasculitis (18.75%). **(Fig. 23a, 23b, 24)**



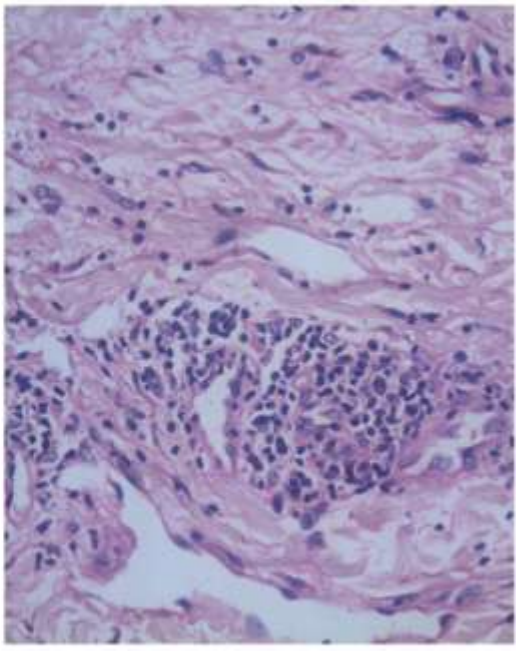
lymphocytic small vessel vasculitis, 200X



mixed small vessel vasculitis, 400X

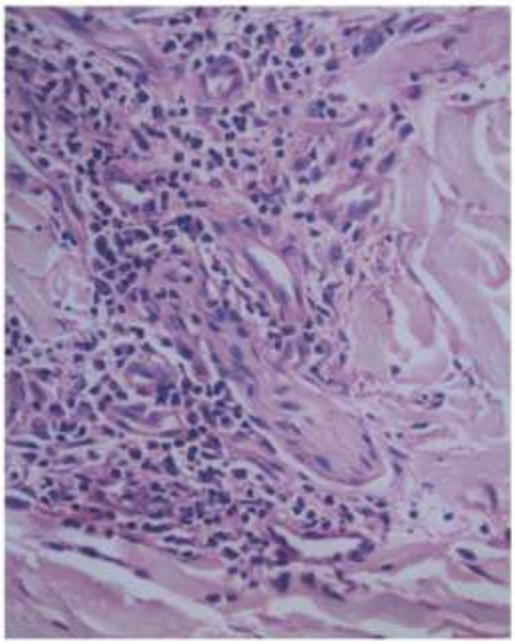


lobular and septal panniculitis, 100X

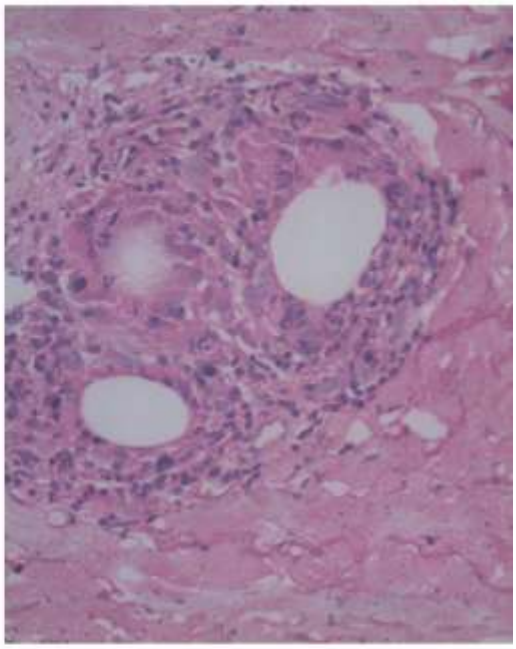


vasculopathic reaction, 400X

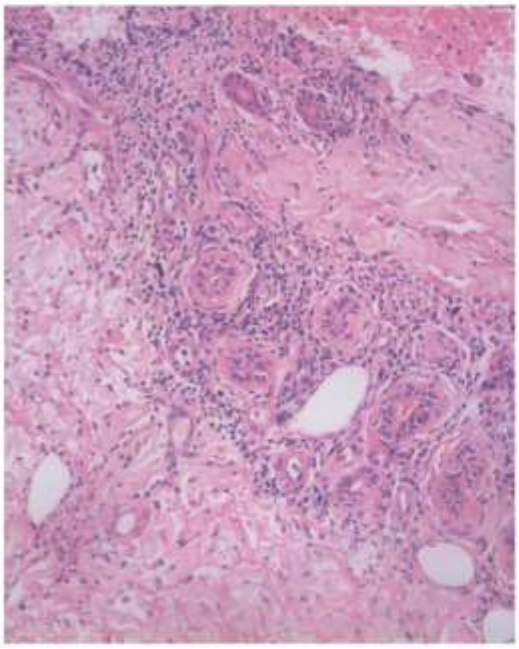
Fig. 23a: Histopathological findings in eschar biopsy specimens



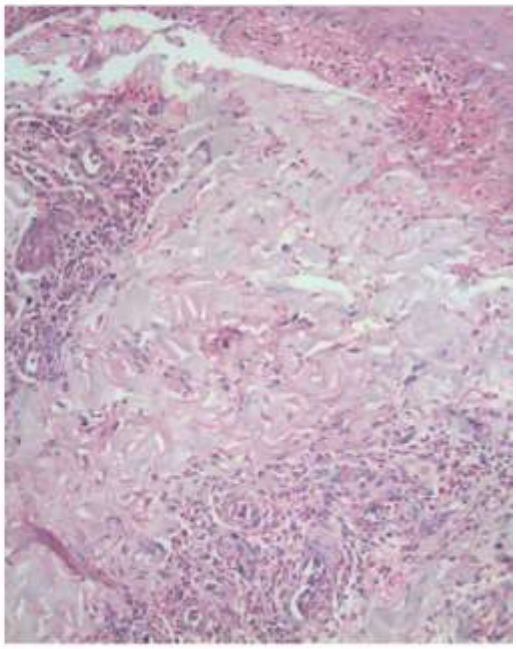
intraneural lymphocytic inflammation, 400X



lipogranuloma, 400X

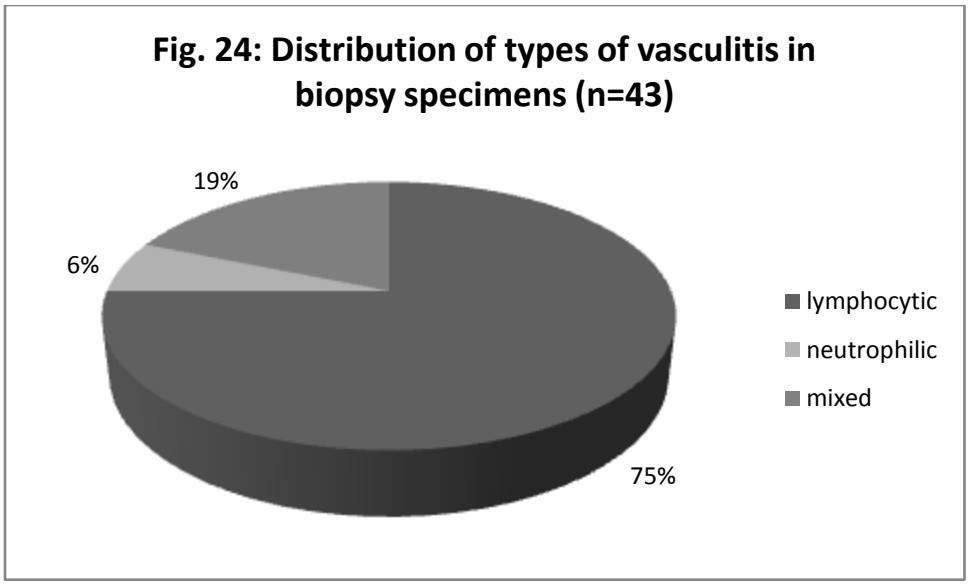


periadnexal lymphocytic inflammation, 200X



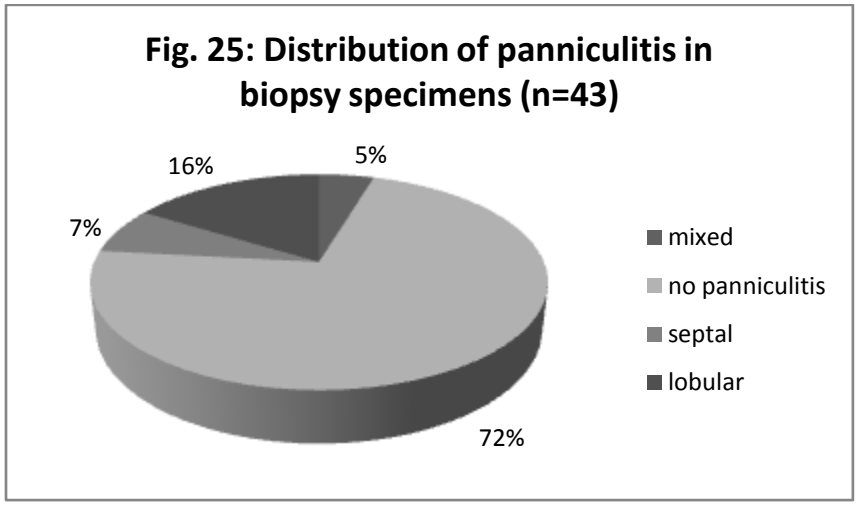
subepidermal cleft

Fig. 23b: Histopathological findings in eschar biopsy specimens



Of the 11 patients who did not have vasculitis, 8 had vasculopathic reaction (18.6%).

Panniculitis was present in 12/43 specimens (27.9%). It was of septal type in 3/12 patients (25%) and lobular type in 7/12 (58.3%) and mixed pattern in 2/12 (16.6%). **(Fig. 25)**



In 3 patients there were features of non-specific inflammation; one of which had peri-adnexal inflammation.

Intra-neural inflammation was seen in 2/43 specimens; one had concomitant lymphocytic vasculitis and the other had vasculopathic reaction.

Granulomatous inflammation was seen in 2/43 specimens; one of which was a lipogranuloma **(Fig 23b);** both had features of vasculitis and panniculitis. Interface changes were seen in 2/43 specimens one of which had sub-epidermal clefting.

42 scab specimens from eschar underwent testing by PCR, 29/42 (69 %) of which were positive whereas 13/42 were tested negative (30.9 %). Among the eschars tested negative for PCR, 10 patients had a positive serology, one had a negative serology while serology was not available for two patients.

COMPLICATIONS

Acute Lung Injury (ALI) / Adult RespiratoryDistress Syndrome (ARDS)

53 patients had respiratory involvement based on history, clinical and radiological findings. ALI / ARDS was present in 33/84 patients (39.2%). Of the patients in ARDS, 4 had Multiple Organ Dysfunction Syndrome (MODS), 3 had shock, 1 had myocarditis, 2 had abortions and 1 died.

CNS involvement

Aseptic meningitis was present in 11/84 patients (13%); one of the patients also had MODS.

Other complications

One had acute pancreatitis and one had splenic abscess. Three pregnant women had contracted scrub typhus in our study period; one had abortion and two had intrauterine death.

The overall mortality rate over one year was 7.38 % (26/352). Among the hospitalized (70/84) patients, one succumbed on the 4th hospital day.

DISCUSSION

There are very few published studies with description of the cutaneous manifestations of scrub typhus in literature most of them from Far East Asia such as Kim et al's study of 176 patients from Korea published in 2007 (12), Zhang et al's study from China on 102 patients from 2006 to 2011 (74) , Ogawa et al's study of 416 patients from Japan in 2001 (78) and Vivekanandan et al's study from Pondicherry India. (58) The rest of the published data with description of skin manifestations are based on small case series and case reports.

Many regions in India have been discovered to harbor *O.tsutsugamushi* in the last few decades. Various pockets in South India have been identified as being endemic for scrub typhus. We conducted this study to describe the cutaneous features of scrub typhus in patients at a tertiary care hospital in Vellore which has been identified as an endemic area with case reports and studies of children and adults from 1997 onwards.

The total number of scrub typhus patients that presented to our hospital from October 2011 to October 2012 was 352. Our study included 84 patients of which 70 were hospitalized while 14 were discharged from emergency medicine after a short observation period. The previous large study from the same centre in Vellore conducted by Chrispal et al over one year from January 2007 to January 2008 included 189 hospitalized patients from the medical wards. (47)

Smaller studies have been done from this region as conducted by Mathai et al in the same centre from October 2001 to February 2002 which included 28 hospitalised patients (18) and a study

by Isaac R et al from October 2002 to January 2003 which was conducted at a community health centre in Vellore that identified 8 patients with scrub typhus. (136)

PLACE

We analysed the endemicity of this zoonotic infection. Most of our patients were from Vellore i.e., 69% while 14.3 % were from Chittoor (n=84). Of the 189 patients in Chrispal et al's study 56.6 % patients from Vellore and 23.8 % patients from Chittoor. Chitoor district is 36 km away from Vellore in the neighbouring state of Andhra Pradesh. This study also demonstrated that patients were found to come from the neighbouring districts of Krishnagiri and Tiruvannamalai inTamil Nadu. Serological evidence of rickettsial infection by positive Weil-Felix test was identified by Kamarasu et al in 2005 in 8/133 patients with acute febrile illness from Tiruvannamalai. However separate data for scrub typhus infection was not available. Patients were also found to come from Cuddapah district in Andhra Pradesh which is nearly 200 km away from Vellore. There is a case report of a single patient from Adilabad district which is the northern most part of Andhra Pradesh bordering Maharashtra. (56)

MONTHLY DISTRIBUTION

Monthly distribution of cases was studied. In the period of one year from October 2011 to October 2012 we found that the peak of scrub typhus cases occurred in the month of October 2011 with 42 cases which amounts to 50% of 84 cases. Chrispal et al's study showed that maximum number of cases from Vellore occurred in the month of September with 24.9 %

(n=189). (47) Mathai et al's study reported that the outbreak of scrub typhus from 2001-2002occurred from the months of October to February. (18) Peak occurrences of scrub typhus was found to preferentially follow the north-east monsoons as (Fig. 8). We had 4 patients in the summer months from April 2012 to June 2012 among the total scrub typhus cases though these were not recruited.

A bimodal distribution of cases has been shown to occur in other endemic countries. Liu et al has reported that in China cases occur throughout the year with a peak of autumn-winter type of scrub typhus during October and the summer type in June-August. (19) In Japan scrub typhus occurs with a peak during November (75) whereas in Taiwan (135) and Korea the maximum number of cases occur from October to November. (137) A smaller proportion of cases have been reported from these areas in the summer months as in May from Japan and Taiwan, April to May from Korea.

AGE DISTRIBUTION

In our study which included only adults, patients belonged to various ages ranging from 18 to 80 years with a mean age of 44.4 ±17 years similar to Chrispal et al's study where the mean age was 45.4 years. (47) The age distribution showed a maximum peak in the 45-55 year age group with a similar distribution in younger age groups (15-25, 25-35 and 35-45 year age groups), all totally accounting for 73.8% of patients (n=84). Our study did not show predilection for any particular age group. There were only 15 cases above 60 years of age which could be due to the fact that this population has restricted activities whereby the risk of acquiring the infection is lower.

Ogawa et al's study had 62 % of cases between ages 51-75 years (n=462). (75) Liu et al' study had 57.9 % of cases between 21-50 years (n=480). (19)

GENDER DISTRIBUTION

In most studies the male: female ratio is similar. In this study the proportion of females which was 58.3 % were more compared to males which was 41.6 % (n=84). Chrispal et al's study included 189 patients with slightly more males at 52.9% and 47 % females. (47) In Singh et al's study from Manipur had males formed 17/38 and females formed 21/38. (50) Kim et al's study had 108 females and 53 males. Liu et al's study included 56 % males and 44 % females (n=480). (19)

Maximum number of females were in the age group of 15-25 years whereas more number of males belonged to the age group of 45-55 years. The sex distribution among various age groups has minimal variations in other epidemiological studies. Zhang et al's study a significant difference was found in the male: female sex distribution among children and adults i.e., between age groups less than or equal to 19 years with 1.6:1 and more than 19 years with 0.9:1 (74) .

OCCUPATIONAL CATEGORIES

Our study included patients from different walks of life that included agricultural/ manual labourers, house-wives and house-maids, auto-drivers, students, salesman, a nurse, office staff including an agricultural officer, unemployed and retired men. All except one who was a

salesman reported direct contact with vegetation. 51.2 % of patients (n=84) belonged to the group that engaged in agricultural activities. Chrispal et al had shown that the major occupational categories consisted of farmers and unskilled labourers who formed 38.8 % while house-wives and unemployed people formed 42. 9 % (n=189).(47) Epidemiological studies done from Japan showed that the largest occupational category was of patients involved in farm work which formed 44 % (n=462). (75) Among all the reported cases of scrub typhus among 1722 cases from China, 84.6 % were farmers (74). Farmers formed the major groups in studies from SriLanka (138), Taiwan (135).

DURATION OF FEVER

In Chrispal et al's study patients were found to have a mean duration of fever of 9.5 days at admission (47). Similar findings were observed in our study where patients had a mean duration of fever of 9.9 days at admission into the hospital. One patient had fever for a maximum of 30 days at admission.

DURATION OF HOSPITAL STAY

The average duration of hospital stay was 5.5 days in Chrispal et al's study (47). We found that the average duration of hospital stay in our study group among the 71 hospitalised patients was of 7.17 days with a maximum of 25 days.

DEFERVESCENCE OF FEVER

Defervescence of fever within 48 to 72 hours of initiation of specific antibiotic is a distinct feature of scrub typhus infection. This entity along with positive serology for scrub typhus was taken as criteria for inclusion into Chrispal et al's study. (47) In 67 / 84 patients we found a mean duration of 1.9 days for the fever to abate with specific therapy which included doxycyline and azithromycin. However 4/67 (6%) patients took longer than 3 days for fever to subside raising the possibility of resistance.

CUTANEOUS FEATURES

ESCHAR

The eschar is the characteristic cutaneous feature of scrub typhus which if present in a patient with acute undifferentiated febrile illness is almost diagnostic of scrub typhus. (82) The pathognomonic eschar has been reported with varying frequencies from endemic areas. Ogawa et al reported eschars in 87 % patients (n=462) from Japan. (75) Various studies from China have shown that eschars occur in the range of 67 to 88 %. (139) (74) (19) Taiwan and Korea have reported similar frequencies. Studies from India have documented the presence of eschar in lesser frequencies compared to higher rates from Oriental countries. Vivekanandan et al reported eschars in 46 % (n=50) in patients from Pondicherry observed from 2006 to 2008. (58) Chrispal et al showed that the frequency of eschar was almost similar at 45.5 % (n=189) in a study done

from Vellore from 2007 to 2008. (47) Our study was able in demonstrate eschars in a higher frequency than reported by Chrispal et al. We had 85.7 % patients with eschars. Better detection by the health care givers in an area known to be endemic could be one reason for the higher rate of eschar in our study. Among the 42 eschar specimens that underwent testing by PCR, 29/42 (69 %) were positive whereas 13/42 were tested negative (30.9 %). Among the eschars tested negative for PCR, 10 patients had a positive serology, one had a negative serology while serology was not available for two patients.

Kim et al's study was the first of its kind that looked at patterns of distribution of eschars in patients with scrub typhus from Korea. (12) This study found a differential pattern of the presence of eschar among the male and female sex which was represented pictorially. Of the 162 patients with eschars, 79.5 % patients had eschars on the anterior aspect of the body. Among males, 35.8 % (n=52) patients were found to have eschars predominantly located below the umbilicus upto 30 cm which included the inguinal area, the perineal areas and the buttocks. However in females the pattern was found to be different with 40.7% (n=108) present over the anterior chest. The earlier study of Ogawa et al on 362 patients of scrub typhus with eschar from Japan had shown that most of the eschars were present over the abdomen and the lower half of the body especially over the feet. They attributed this pattern to the fact that the lower part of the body was more accessible for the mite to feed on. (78) Zhang et al reported that the anterior chest, superior aspect of the abdomen and the axilla were common sites for the presence of eschar. (74)

There are only two studies that have reported the site wise distribution of eschar from India. Vivekanandan et al reported eschars occurring commonly in the axilla, breast and groin.(58)

There are many isolated case reports of description of eschars in scrub typhus from different parts of India. Atypical sites such as over the forearm near the wrist joint have been reported in one patient from Shimla by Aggarwal et al. (67) The other sites reported as atypical by the same author such as near the elbow joint, the dorsum of the penis and the medial aspect of the thigh have been reported in the aforementioned Kim et al's study . Singh et al reported the occurrence of 55 % of eschars in the perineal area, 37 % over the upper torso and 8 % over the lower abdomen and thighs among 38 patients from Manipur. (50)

Our study showed the distribution of eschars to be 45.8% on the trunk, 15.3% on the lower limbs, 12.5% in the axilla, 8.3% in the groin, 6.9% over the genitalia, 5.5% on the neck and 5.5% on the upper-limb. 63/72 (87.5%) eschars were present over the anterior aspect of the body and 9 (12.5%) were present over the posterior aspect of the body. Among the 9 eschars over the posterior trunk 7 were in females while 2 were in males.

Not all patients in our study had the typical necrotic scab of an eschar. 21/72 patients were found to have well defined ulcers that appeared punched out with a rim of erythema. Maximum number (7/21) was located over the inner aspect of the thigh. It is possible that ulcers occur preferentially in areas with maceration. (12) Many patients reported having noticed the falling off of the scab on subsequent days once the eschar was brought to their attention. Among Asians clothing patterns differ markedly among the sexes. Kim at al suggested that the warmth and occlusion in the mammary areas in females due to tight inner wear and due to the use of tight under wear in males could be the possible factor leading the preferential distribution among the sexes .(12) It is possible that the migration of the mite along the body surface gets restricted at these areas where it settles for a blood meal.

Zhang et al also noted that the average diameter of the eschar was 7.05 (+/-4.23) mm. Other studies do not give a recording of the size of the eschar though Kim et al describes that a typical eschar ranges from sizes 5 to 20 mm. (12) Our study showed that eschars ranged from as small as 3x2 mm to as large as 25 x9 mm. Based on size there were 15 eschars (20.83 %) out of the 85 eschars which were less than 5mm. The average diameter of an eschar was found to be 6.5 mm in males and 7.5 mm in females among the 72 patients with eschar in our study.

There was one patient with double eschars who had a positive serology for scrub typhus and another patient with three eschars that were grouped in nature.

Confirmation of multiple eschars in these patients as being conclusively due to the bite of the trombiculid mite was not done. PCR for O.tsutsugamushi would have been the ideal confirmatory test on the eschar.

In our study of 84 patients, the pathognomonic eschar was absent in 12 patients. These patients had a positive serology for scrub typhus by IgM ELISA .

RASH

Our study revealed the presence of an erythematous papular rash in a single patient among the 84 cases. This patient also had an eschar. However histopathological examination of this rash was not done due to lack of consent.

Studies from Japan, Korea, Taiwan and China show a high occurrence of a generalized rash.

However studies from India suggest that rash occurs rarely **(Table 6)**. The earliest study from Vellore by Mathai et al showed the presence of rash in 22 % of 28 patients. (18) Chrispal et al' study revealed the presence of a rash in 5.8 % of patients. Rash was rarely reported from Jammu. (72) There was no rash reported in 38 patients from Manipur. (50) The reasons for rarity of rash in patients from India are not known. It could be possible that genotype variation could result in variability of clinical features as was shown by Kim et al's study which showed that the occurrence of eschar and rash was higher in patients infected with the Boryoung strain than the Karp strain. (115)

Our study did not show any other cutaneous features.

Table 6: INCIDENCE OF ESHCAR AND RASH IN SCRUB TYPHUS IN VARIOUS STUDIES

Place, author	**Number of patients**	**Eschar**	**Rash**
Japan, Ogawa et al	462	87 %	93 %
China, Zhang et al	102	86.3 %	68.6 %
China, Zhang et al	104	67.3 %	52.9 %
China, Liu et al	480	88.5 %	90.4 %
Korea , Kim et al	176	92.04 %	not available
SriLanka, Liyanapathirana et al	168	55-67 %	10-36 %
India (Vellore), Mathai et al	28	not available	22 %
India (Vellore), Chrispal et al	189	45.5 %	5.8 %
India (Pondicherry)	50	46 %	not available

HISTOPATHOLOGY OF ESCHARS

We attempted to characterize the salient histopathological features. Among the 72 patients who had eschars, 43 patients gave consent for undergoing a biopsy.

Histopathological examination of rash which had developed in one patient was not performed due to lack of consent. There are very few studies in literature of histopathological features of eschars. The earliest available study on biopsy features of eschars was done by Allen et al in 11 patients published in 1945. (70) Paris et al had studied 14 eschar biopsy specimens. (16) Kim et al studied 22 eschar specimens. (123)

We were able to demonstrate the following features.

Ulceration, which is a feature that corresponds to what lies beneath the necrotic scab, was seen.

24/ 32 (75 %) patients had evidence of lymphocytic vasculitis. There were two reports of isolated leucocytoclastic vasculitis among 32 patients (6.25 %). Six samples of the 32 specimens demonstrated features of a mixed vasculitis (18.75 %). Paris et al demonstrated leucocytoclastic vasculitis in one sample of the eschar . Neutrophilic vasculitis has also been noted by Kim et al. (123)

We were able to demonstrate panniculitis in 12 of the 43 samples of eschar (27.9%).

Two specimens of 12 had features of both septal and lobular panniculitis (16.6%) whereas predominantly lobular panniculitis was seen in 7 patients (58.3%). Predominantly septal panniculitis was seen in 3 samples out of the 12 samples of panniculitis (25 %). Paris et al had noted superficial panniculitis in their biopsy specimens. Lobular or septal panniculitis with

predominant mononuclear cell infiltrates had been noted by Kim et al.(123) Allen et al do not mention panniculitis in their study.

Vasculopathic reaction without a vasculitis was seen in 8 specimens of the 43 eschar samples (18.6 %).

We were also able to demonstrate intraneural involvement as seen in two patients. Focal peri-adnexal inflammation was noted in one patient. Paris et al had demonstrated perifollicular, periglandular and focal perineural inflammation in eschar biopsies. (16) Periappendageal mononuclear inflammation has been observed by Allen et al. (70)

Other features that have not been documented previously in literature are the following. Granulomas and granulomatous inflammation including a lipogranuloma was seen in two patients. Allen et al had observed foreign body giant cells around a mite particle in one eschar specimen in his study.
Interface changes were seen in two samples. Sub-epidermal blistering as noted by Paris et al was seen in one of our specimens with interface change.

LYMPHADENOPATHY

Lymphadenopathy was noted only in 15 of the 84 patients. Regional lymphadenopathy with respect to the eschar was noted in 10 / 72 patients (13.8%) while generalized lymphadenopathy was noted in 5/85 patients (6%).

There was no evidence of tender lymphadenopathy in our study as mentioned in other series. Chrispal et al had reported lymphadenopathy in 6.3 % (n=189) which was not separately classified as regional or generalized lymphadenopathy. Studies from the Far East report higher incidence of lymphadenopathy. Ogawa et al reported lymphadenopathy in 51 % patients (n=416) of which majority i.e., 75% were regional. (78) Tsay et al reported lymphadenopathy in 33 % (n=33). (140)

LABORATORY PARAMETERS

In our study the commonest deranged laboratory parameter was elevation of the liver enzyme SGOT/ AST which was seen in 79/84 (94 %) patients while SGPT was elevated in 67/84 (79.7%) as in most studies. Ogawa et al reported elevation of SGOT in 85 % patients whereas SGPT elevation was seen in 78 % patients. (78) Chrispal et al reported that among 189 patients SGOT was elevated in 95.2 % patients and this was more commonly seen than elevation of SGPT. (47) Tsay et al reported 81 % elevation of SGOT and SGPT elevation in 75 % (n=33) . (140) Hu et al reported elevation of SGOT in 89.3% patients and that of SGPT in 91.7 % patients (n=30).(95)

The mean white blood cell count was 11,469 /mm^3. Leucocytosis was present in 38/84 patients (45.2%), whereas 2 of our patients had leucopenia. Chrispal et al reported leucocytosis in 37 % (n=189) patients. Tsay et al noted leucocytosis in 34 % patients (n=33) whereas leucopenia was noted in 19 % (6/32) patients.

Thrombocytopenia was noted in 53/84patients (63%). Chrispal et al reported thrombocytopenia in 70.7% patients (n=189). Tsay et al reported low platelet counts in 44 % (14/32).(140)

COMPLICATIONS

ARDS /ALI was found in 33 / 84 patients (40%). Chrispal et al's study had shown 24.9 % (n=189) of ARDS. Tsay et al had shown 15 % (n=33) cases of ARDS from Taiwan. Wang et al had shown ARDS in 8/72 patients and a mortality rate of 25 % in them.(141)

Aseptic meningitis was seen in 11/84 patients (13%) and one of these had multi organ dysfunction syndrome. Chrispal et al had shown 20.6 % patients with aseptic meningitis (n=189). Our rates were similar to other studies. Meningitis has been found to occur in 5.7% to 13.5 % in various studies from the Far East. (142)

Among the other severe complications we had one patient with acute pancreatitis and another patient with splenic abscess both clinically and radiologically. These findings were not present in the group of patients in Chrispal et al's study. Myocarditis occurred in 2/84 patients. Chrispal et al had reported myocarditis in 2/189 patients. Four patients (4.7 %) had presented in shock to emergency medicine requiring admission to intensive care units. Chrispal et al's study had 13.8% (n=189) patients with hypotension.

Of the 84 patients, four patients were documented to have multiorgan dysfunction syndrome all of whom recovered with specific treatment and supportive management. MODS has been reported in many case reports. (112) (143) However there were no large series of the same.

We had three antenatal patients all of whom had a bad outcome in relation to pregnancy. One patient had an abortion at 3 months of gestation. The other two patients developed intrauterine death at term. There was one patient who had delivered a normal term child elsewhere and was

hospitalised subsequently with severe scrub typhus. Mathai et al reported 3 stillbirths and 1 abortion among 5 pregnant patients from Vellore. (48)

There was one patient among the 84 in our study group who developed uveitis which resolved at the time of discharge. One of the earlier studies by Scheie on ocular changes in 451 patients of scrub typhus had revealed uveitis in 1.3 %. (108) Uveitis has not been commonly reported.

MORTALITY

One patient among our 84 study patients succumbed. However among the total number of 352 patients seen in our institution over one year 7.38 % (26 /352) died. The mortality rate seems to have reduced considerably from the earlier studies from the same centre. Mathai et al had reported a mortality rate of 10.7 % among 28 patients. Varghese et al reported a mortality rate of 14 % (n= 207) whereas Chrispal et al had shown that the mortality rate was 12.2 % in their study among 189 hospitalised patients. This would reflect earlier and better detection rates of scrub typhus among patients with acute undifferentiated febrile illness with a probable consequential decrease in mortality.

We attempted to correlate severity of scrub typhus with absence of eschar. Kim et al had shown that absence of eschar was associated with severe clinical features.(76) We found that there was no statistical difference between duration of fever and hospitalization, presence of complications of scrub typhus and various laboratory parameters like total WBC counts, platelet count, and SGOT, SGPT and creatinine levels when comparing patients with and without eschar

CONCLUSIONS

1. The peak distribution of cases occurred in the month of October corresponding with the north east monsoons.

2. Majority of patients (51.2%, 43/84) engaged in agricultural activities.

3. In our study of 84 patients with scrub typhus, cutaneous lesions were found in 85.7 % (72/84) patients all of whom presented with the pathognomonic eschar.

4. 29.1 % (21/72) of patients with eschar presented as discrete ulcers without the necrotic scab

5. The eschar was found to have a mean size of 7.1±4.5mm and 20.8 % (15/72) eschars were less than 5mm in size

6. More eschars were found over the anterior aspect of the body (63/72) and significantly larger eschars (p= 0.048) were found over the neck and groin with a mean size of 11.5±6.5mm.

7. There was no significant differential distribution of eschars among males and females

8. One patient with eschar had a concomitant papular rash

9. Histopathological examination of the eschar revealed vasculitis in 32/43, vasculopathic reaction in 8/43 and 3 patients with non-specific inflammation one of which had peri-adnexal inflammation. Of the patients with vasculitis 75 % (24/32) had lymphocytic vasculitis, 6.25% (2/32) had leukocytoclastic vasculitis in, 18.75% (6/32) had mixed vasculitis, Panniculitis was seen in 27.9% (12/43) specimens which were mostly lobular ; 58.3% (7/12). Rare findings of intraneural inflammation, interface changes and granulomatous inflammation were observed.

10. Defervescence of fever with antibiotics was found to be delayed in 4/67 patients.

11. Leukocytosis was found to be significantly more in patients with complications (p value = .001).

LIMITATIONS

1. All patients with eschars did not consent for biopsy.
2. Patients with mild symptoms who were presumptively treated with specific therapy based on the presence of eschar could not be included in the study as they would get discharged from emergency medicine in a day.
3. Serology for IgM ELISA for scrub typhus was not tested in few patients.

SUMMARY

OBJECTIVES:

To describe the skin manifestations, clinical profile and the histopathology of skin lesions in patients with scrub typhus.

METHODS:

A cross-sectional, observational study was performed in a tertiary hospital in Vellore, an endemic area for scrub typhus. Among adult patients with a febrile illness of 5 – 28 days duration and either a positive serum scrub typhus IgM ELISA or with the presence of an eschar 84 patients were recruited from October 2011 to October 2012.Eschar size and distribution of skin lesions, demographic details and clinical features were recorded. The eschar was biopsied for histopathological examination. Data was analysed using chi-square test, Fisher's exact test and Student's t-test.

RESULTS:

Patients with scrub typhus from Vellore constituted 58/84 of the study patients while the remaining were from the neighbouring districts of Tamil Nadu and Andhra Pradesh. The mean age was 44.4 ±17 years. Agricultural labourers formed the major group of with 51.2 % (43/84). Average duration of fever at presentation to hospital was 9.9±4.4 days. Eschars were found in 85.7 % (72/84) of the study patients, one of whom had a concomitant rash.

Maximum number of eschars; 45.8% (33/72) were located over the trunk followed by the lower limbs (11/72). The flexural regions like the axilla (9/72), groin (6/72), genitalia (5/72), neck

(4/72) and upper-limbs (4/72) were less frequently involved. Most of the eschars (87.5 %, 63/72) were found over the anterior aspect of the body. Discrete ulcers without the overlying necrotic scab were noted in 21/72 patients. These were found mainly in the inner aspect of the thigh (7/21) followed by the axilla (3/21), scrotum (2/21), breast (2/21), chest wall (2/21), 1 each over the groin, perineal area, inner aspect of the upper arm and the lumbar area. There was no significant difference in gender-wise distribution of eschar. The mean eschar size was 7.1±4.5mm. Eschars less than 5mm were seen in 15/72 (20.8 %) patients. The largest eschars were over the groin and neck which was statistically significant ($p<0.05$). Absence of eschar did not correlate with severe scrub typhus. The following clinical features were noted. Regional lymphadenopathy in 13.8 % (10/72), generalized lymphadenopathy in 6% (5/84), leucocytosis in 45.2% (38/84), elevated SGOT in 94% (79/84), elevated SGPT in 67/84 patients (79.7 %), thrombocytopenia in 63 % patients (53/84), elevated creatinine in 23/84 patients (27.3%), respiratory complications in 39.2 % (33/84), aseptic meningitis in 13 % (11/84). Most patients responded to specific antibiotic therapy. However, in 4/ 67 patients, defervescence took more than 72 hours. Histopathological examination of the eschar revealed vasculitis in 32/43, vasculopathic reaction in 8/43 and non-specific inflammation in 3 of which had one had peri-adnexal inflammation. Of the patients with vasculitis 75 % (24/32) had lymphocytic vasculitis, 6.25% (2/32) had leukocytoclastic vasculitis in, 18.75% (6/32) had mixed vasculitis, Panniculitis was seen in 27.9% (12/43) specimens which were mostly lobular ; 58.3% (7/12), 3/12 (25%) had septal panniculitis whereas 2/12 (16.6%) had septal and lobular panniculitis. Rare features like intraneural inflammation was seen in 2/43 , granulomatous inflammation including a lipogranuloma was seen in 2/43 , interface changes in 2/43 with sub-epidermal cleft in one of them were seen.

CONCLUSIONS: Our study population of pts had a higher occurrence of eschar but a low frequency of rash. Eschars may not always present with a necrotic scab. Larger eschars were located over the neck and groin. Eschars were most frequently located over the trunk. Absence of eschar was not associated with risk of complications. Leucocytosis was found to be more in patients with complications. Delayed defervescence with specific antibiotic therapy of fever was noted in few patients. Lymphocytic vasculitis and panniculitis were the common histopathological findings in eschar biopsies.

RECOMMENDATIONS

1. Careful search for an eschar however small, in a patient with acute febrile illness should lead to a suspicion of scrub typhus infection in an endemic area especially in the cooler months of the year is vital as specific therapy is available and is effective.

2. The differences in cutaneous manifestations observed in this study as compared to studies from Far East Asia might be related to varied genotypes and studies should be done to identify the strains in this endemic area.

3. As some of our patients hailed from areas in Tamil Nadu and Andhra Pradesh where scrub typhus has not been reported earlier, epidemiological studies need to be done.

4. Antibiotic susceptibility patterns need to be studied especially in patients with delay in defervescence of fever.

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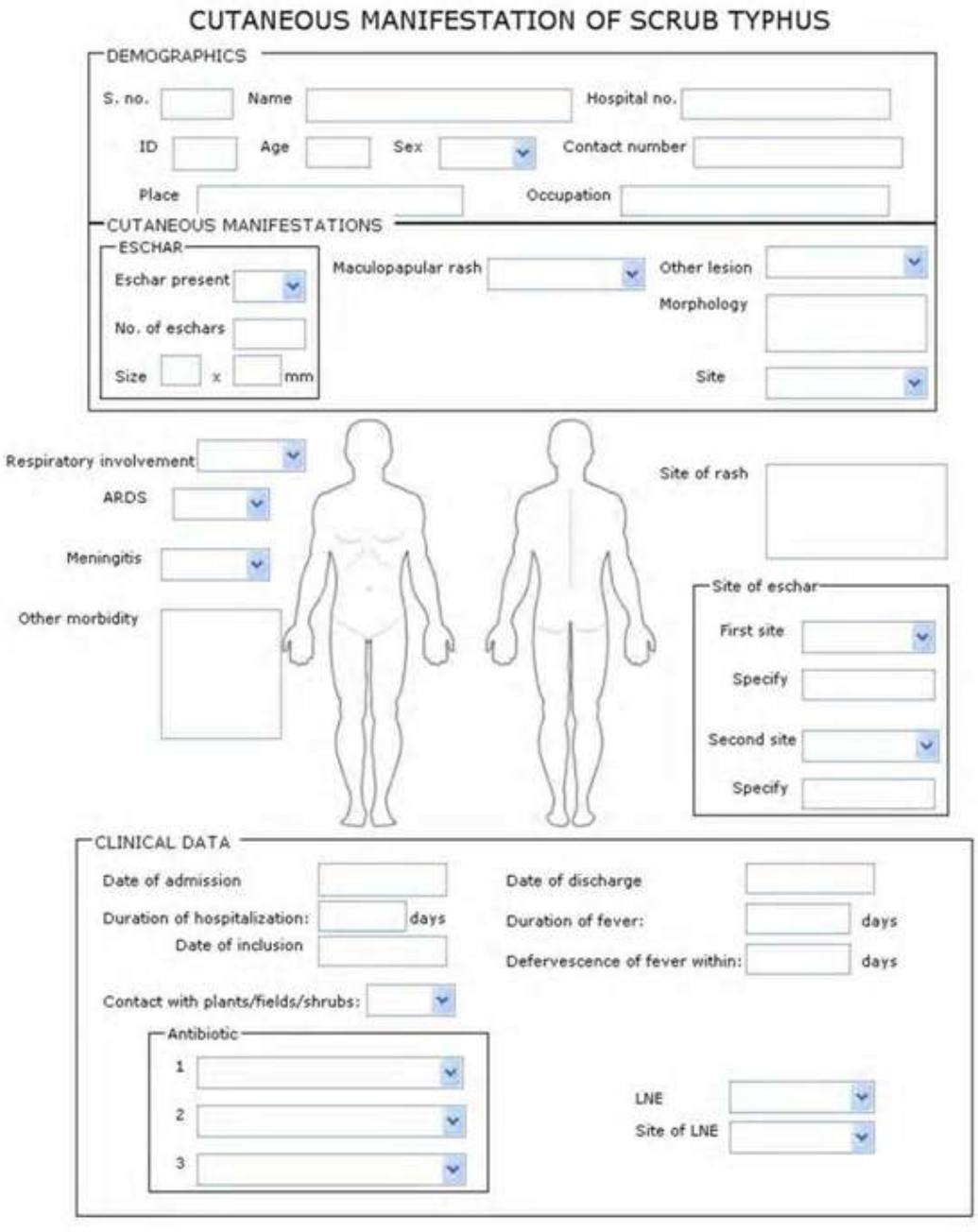
APPENDIX - I

LIST OF ABBREVIATIONS

MICU	Medical Intensive Care Unit
MHDU	Medical High Dependency Unit
ELISA	Enzyme Linked Immunosorbent Assay
PCR	Polymerase Chain Reaction
SGOT	Serum glutamic oxaloacetic transaminase
AST	Aspartate transaminase
SGPT	Serum glutamic pyruvic transaminase
ALT	Alanine transaminase
O. tsutsugamushi	Orientia tsutsugamushi
ARDS	Acute respiratory distress syndrome
IHC	Immunohistochemistry
H&E stain	Haematoxylin and eosin
MODS	Multiple Organ Dysfunction Syndrome

APPENDIX – II

PROFORMA



Name

INVESTIGATIONS

Scrub typhus IgM ELISA ☐ In lab

IHC

Total WBC

DC N E M L B Shift to left

Platelets

Creatinine

LFT TB DB SGOT SGPT

Histopathology

Lesion Lymphocytic vasculitis

HPE

vasculitis panniculitis

type of vasculitis vasculopathy

2

APPENDIX – III

Patient Information sheet

Study Title:

'Cutaneous manifestations of scrub typhus '

Purpose of research:

There is a possibility of an infectious disease in the patient which is caused by a bug that infects humans through the bite of a mite. It leads to fever, a scab at the site of the bite, a rash and organ involvement to various extents depending on the type of the bacteria.I am doing a study to describe the skin features of this disease and to biopsy the skin lesion(scab or rash or both if present) to study it's features.

Expected duration of the Subject's participation:

From admission in the ward /medical high dependency unit/ intensive care unit till discharge

Description of the procedures:

This study would involve taking biopsy of the scab/ or rash following adequate local anaesthesia. There may be one or two stitches at the biopsy site which will be removed after one week. Clinical photographs of the skin lesions may be taken.

Risks or discomforts to the Subject:

As the study does not include any trial treatment, there is no extra risk for the patient due to participation in study and there will not be any additional cost of treatment for the patient

Benefits to the Subject:

There may not be any benefit to the participant.

Benefits to others:

Information gathered from this study might help in understanding the disease better which could lead to better diagnosis and early treatment.

Confidentiality:

The patient's identity will not be revealed in any form or released to third parties or published. Only the investigators of this study will be able to access the Subject's medical records

Participation:

The patient's participation in the study is entirely voluntary and is free to withdraw at any time, without giving any reason. Refusal to participate or withdrawal will not involve any penalty or loss of benefits to which the Subject is otherwise entitled

நோயாளி :

ஸிகரட் பையிலில் தோலில் தொண்டும் அரிக்கும் பிரச்சனை கொண்டு ஆய்வுக்கு வந்தார்.

நோயாளிக்கு இருக்கும் ஒரு நோயாளி ஒரு மனிதன் மனைவியை இழந்து, அந்த இரண்டாவது திருமணம் இருக்கவும், இந்து கொள்கையில், மனைவி இறந்த உடலில் பால், தயிர் மற்றும் இருவரின் வலதுபுறம் அடையாளம் பல இருப்பதாகவும் நம்பிக்கை.

நான் இந்த நோயாளி தோலில் தொண்டும் அரிக்கும் பிரச்சனை, கொண்டை ஆய்வதை பற்றியும் இந்த ஆராய்ச்சியின் முடிவு விளக்கிக்கொண்டு இந்த ஆராய்ச்சி ஒத்துழைத்தால், இந்த எல்லை வரைக்கும் இந்த நோயாளியை தினசரி பிரச்சனை இல்லாமல் இருக்கலாம்.

பங்கேற்பாளர் எதிர்பார்க்கப்படும் கால அளவு :

பங்கேற்பாளர் மருத்துவ மனையில் ஒரு மின்விசிறி அல்லது தீவார சிகிச்சை மின்விசிறி தேர்ந்தெடுத்து அறையில் பயன்.

தலைமையாசிரியர் விளக்கம் :

இந்த ஆராய்ச்சியில் மொத்தமாக ஒரு இடத்தில் மனிதக் கருத்து எலும்புத் துண்டுகள் கண்டுபிடிக்கப்பட்டது. அந்த இடத்தில் 1 அல்லது 2 எலும்புகள் மொத்தமாகக் கிடைத்தன. மண்ணால் ஒரு எலும்பு முற்றிலும் மூடப்பட்டிருந்தது. தொல்லியல் துறை கருவிகளைக் கொண்டு அவை அகழ்ந்தெடுக்கப்பட்டன.

பரிந்துரைகளை ஏற்கும் அதிகாரிகள் :

இந்த ஆராய்ச்சியினால் பரிந்துரைகளை ஏற்கும் அதிகாரிகள் யார் என்றால், சுகாதாரத்துறை அமைச்சகம் மற்றும் தொல்லியல் ஆய்வுத்துறை.

பரிந்துரைகளுக்கான காலவரம்பு :

பரிந்துரைகளை ஏற்கும் இந்த தொல்லியல் கள ஆய்வு முடிவுகளை ஏற்கும் காலவரம்பு :

இந்த அறிவிப்பு நாளன்று தொடங்கி ஒரு வருடத்திற்குள் இந்த தொல்லியல் கள ஆய்வு முடிவுகளை ஏற்கும் முடிவுக்கு வரவேண்டும்.

முடிவுரை :-

பரிந்துரைகளை ஏற்கும் இந்த தொல்லியல் ஆய்வுகளை ஏற்கும் அதிகாரிகள் தெரிவிக்கும் கருத்துகள், ஆய்வாளர்கள் மற்றும் பரிந்துரைகளை ஆவணப்படுத்தல் அவசியம்.

ஆராய்ச்சியாளர் :

மருத்துவர் விஜயா ஜெரி, நோய் சிகிச்சை மனநல சிறப்பு, வேலூர்.

பங்கேற்பு :

நீங்கள் தானாக முன்வந்து இந்த ஆராய்ச்சியில் பங்கேற்கலாம். எந்த நேரத்திலும் எந்த காரணத்தையும் சொல்லாமல் விலகிக்கொள்ளலாம். ஆய்வாளருக்கு வேறு ஏதாவது, மறுபரிசீலனை பங்களாருக்கு எந்த ஒரு இடர்பாடோ அல்லது அவருக்கு எனக்கும்பட்டும் சிகிச்சையிலோ எந்த பாதிப்பும் ஏற்படாது.

பங்கேற்பாளரின் தேதியுடன் கையொப்பம் } - 96
எண்ணிக்கை

APPENDIX – IV

Informed Consent form

Study Title: Cutaneous manifestations of scrub typhus

Subject's Name:

Date of Birth / Age:

Please initial box

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that the Sponsor of the clinical trial, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s) [ ]

(v) I agree to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative:
Date:
Signatory's Name:

Signature of the Investigator:
Date:
Study Investigator's Name:

Signature of the Witness:
Date:
Name of the Witness:

శీర్షిక/ అధ్యయనము :- "Scrub Typhus వల్ల వచ్చేవ్యాధి పై వ్యాధి నిర్ధారణ కొరకు పరీక్షలలో, వ్యాధి నిర్ధారణ కొరకు రక్తనమూనా ఇవ్వడానికి అంగీకారం

Subject name :-

Date of birth /Age :-

Please Initial box
(subject)

1. నాకు ఇవ్వబడిన సమాచారమును నేను చదివి (లేదా) నాకు చదివి వినిపించగా, ఈ అధ్యయనం గురించి నాకు అర్థమైనది. అందుచే ఈ అధ్యయనంలో నన్ను చేర్చుకొనుటకు అనుమతిస్తున్నాను.
2. ఈ విషయమునకు నా స్వేచ్ఛాయుతముగా సమ్మతిస్తున్నాను. ఎప్పుడయినా, ఏ కారణమును లేకుండా కూడా అధ్యయనం నుండి వైదొలగే విషయంలో కూడా నేను చేర్చబడ్డ కలదు (లేదా) అంగీకరిస్తున్నాను.
3. నేను, క్లినికల్ సంబంధిత పరిశోధన సంబంధ విషయాలలో వివరించుటకై, అధికారులు పరిశీలించుటకు మరియు పరీక్షలకు సంబంధించిన వ్యాధిని, వ్యాధి తీవ్రతను చదివే వివరాలు, వైద్య వివరాలు పరిశీలించే కొరకు, వివరాలను అధికారులకు నా అనుమతి అవసరమని నేను (లేదా) అంగీకరిస్తున్నాను. నా యొక్క గోప్యతకు భంగం కలుగకుండా జాగ్రత్తలు చేయబడతాయి, మరియు వ్యాధికి చికిత్స చేయు సమయంలో కాని, (లేదా) చేయకపోయిన కాని ఇవ్వకపోయిన (లేదా) నిలిపివేసిన, ఎలాంటి నష్టం లేదని తెలిపి.
4. ఈ విషయము యొక్క నా పాత్ర పరిశోధన యొక్క ఫలితములను, వ్యాధి సంబంధ వైద్య విషయాలను ప్రచురించుటకు కావలసిన అనుమతులు చేసి అంగీకరిస్తున్నాను.
5. ఈ విషయము (study) లో పాల్గొనుటకు నేను అంగీకరించు చున్నాను.

సంతకము చేసిన వేలిముద్ర
(Signature or thumb impression of the subject or legally)

Acceptable Representative] తగిన ప్రతినిధి సంతకం (లేదా వేలిముద్ర) —

పునరుద్ధరణ పేరు (Signatory's name) —

పరిశోధకుని సంతకం (Signature of the investigator) —

(Date) తేదీ —

పరిశోధకుని పేరు (Study Investigator's name) Signature of the witness —

సాక్షి సంతకం (Signature of the witness)

Date —

సాక్షి పేరు Name of the witness —

APPENDIX – V

Master Chart

S2	name	hospital2	age	sex	PCR	Dateofinclusion	complication	Resp	ARDS	Meningitis	Othermorbidity
1	Sundaresan D.	038224F	55	1	2	10/1/2011	1	1	1	2	
2	Parivallal	545645C	29	1	1	10/1/2011	2	2	2	2	
3	Anjali R.	039219F	50	2	1	10/4/2011	2	2	2	2	
4	Vijayalakshmi	426182D	41	2	1	10/5/2011	2	2	2	2	
5	Udhayashankar	039393F	41	1	2	10/5/2011	2	1	2	2	uveitis
6	Pachiammal	046203F	75	2	1	10/5/2011	2	2	2	2	
7	RAVINDRAN	046277F	29	1		10/5/2011	2	2	2	2	
8	Ramakrishna	046273f	30	1	1	10/6/2011	1	1	1	2	
9	Santhya	464719d	23	2		10/6/2011	2	2	2	2	
10	Mani	044792f	60	1		10/6/2011	1	1	1	2	MODS
11	Nirmala	044081f	31	2	2	10/6/2011	1	1	1	2	MODS, abortion
12	Jeeva	039433f	25	2		10/7/2011	1	1	1	2	
13	Chandrakalavath	046400F	45	2	1	10/7/2011	2	2	2	2	
14	Ammu	942942D	22	2		10/7/2011	1	1	1	2	
15	Sharma	039395F	28	1		10/8/2011	1	1	1	2	
16	Vasanthapriya	048145F	27	2	1	10/8/2011	2	2	2	2	
17	Sarojamma	050014F	67	2	1	10/8/2011	2	2	2	2	
18	Easwaramma	050104F	22	2		10/10/2011	2	2	2	2	
19	Chinama	050087F	75	2	1	10/10/2011	2	1	2	2	
20	Nirmala	050533F	33	2	2	10/10/2011	2	2	2	2	
21	Santhikumari	046305F	19	2		10/10/2011	1	1	2	1	
22	Rajasekhar	046106F	23	1	1	10/11/2011	2	2	2	2	
23	Perumal	046070F	80	1		10/11/2011	1	1	1	2	shock
24	Shenbagavalli	039430F	20	2		10/11/2011	1	1	1	2	
25	Selvi	050080F	40	2	1	10/11/2011	1	2	2	1	
26	Kumar	050096F	18	1		10/12/2011	2	1	2	2	
27	Nagammal	046473F	68	2		10/12/2011	1	1	1	2	shock
28	Farzana	047905f	24	2		10/14/2011	1	1	1	2	myocarditis
29	Lokesh	050069F	18	1		10/14/2011	1	2	2	1	
30	Thilagavathy	054005F	45	2	1	10/14/2011	1	1	1	2	
31	Hasmath Bee	050031F	55	2		10/12/2011	2	2	2	2	
32	Anitha	050102F	28	2		10/15/2011	1	1	1	2	IUD
33	Kodi Reddi Rani	046077F	55	2	1	10/17/2011	1	1	1	2	
34	Murugan	050259F	45	1	1	10/18/2011	1	1	1	2	
35	Muniyamal	050170F	60	2	2	10/18/2011	1	1	2	1	
36	Lalitha	054117F	56	2	1	10/19/2011	2	2	2	2	
37	Govindaraj	054167F	60	1		10/22/2011	1	2	2	1	
38	Sharmila	050272F	24	2	1	10/20/2011	1	1	2	2	IUD
39	Chandran	054109F	35	1	1	10/21/2011	1	1	1	2	
40	Valli	038822F	24	2		10/21/2011	1	1	2	1	
41	Ramamoorthy	055303F	45	1	2	10/22/2011	2	1	2	2	
42	Jayalakshmi	054475F	61	2	1	10/24/2011	2	2	2	2	
43	Vasantha	054660F	50	2		11/2/2011	2	1	2	2	
44	Manjula	054499F	39	2		11/1/2011	1	1	2	2	acute pancreatitis
45	Chinnapappa	064279F	57	2	2	11/1/2011	1	1	1	2	shock
46	Karunakaran	064183F	35	1	2	11/2/2011	2	1	2	2	
47	Velayutham	046107F	29	1		11/2/2011	1	1	1	1	MODS
48	Valliammal	064567F	60	2	1	11/9/2011	2	1	2	2	
49	Jagandhan	064491F	50	1	1	11/9/2011	1	1	1	2	
50	Selvi	059192F	47	2	1	11/3/2011	1	1	1	2	
51	Kalavathi	065797F	36	2	1	11/10/2011	1	1	2	1	
52	Perumal	022388B	54	1	2	11/12/2011	2	2	2	2	
				1=male 2=female	1 = pos 2= neg		1=yes 2=no	1=yes 2=no	1=yes 2=no	1=yes 2=no	

S2	name	hospital2	age	sex	PCR	Dateofinclusion	complication	Resp	ARDS	Meningitis	Othermorbidity
53	Rathanamammal	069823F	72	2	2	11/12/2011	1	1	1	2	death
54	Amala	074982F	66	2		11/17/2011	1	1	1	1	
55	Vasantha	066027F	55	2		11/17/2011	1	1	1	1	
56	Subramani	068428F	71	1		11/17/2011	2	2	2	2	
57	Bhagavathi	307744D	29	2		11/26/2011	2	2	2	2	
58	Routh	077312F	45	2		11/26/2011	2	1	2	2	
59	Lakshmi	077425F	47	2		11/26/2011	1	1	1	2	
60	Radha	565087B	65	2	1	11/26/2011	1	1	1	2	
61	Palani	082664F	45	1	1	11/24/2011	1	1	2	2	shock
62	Duraiswamy	079364F	73	1	2	11/26/2011	2	1	2	2	
63	Ramesh	076352F	37	1		11/26/2011	1	1	1	2	
64	Balasundaram	077417F	46	1		11/24/2011	2	1	2	2	
65	Varada Raj	077495F	60	1	2	11/26/2011	1	1	1	2	
66	Saraswathi	077491F	23	2		11/29/2011	1	2	2	1	
67	Latha	085301F	25	2	1	12/3/2011	2	1	2	2	
68	Maickam	085350F	52	1		12/3/2011	2	2	2	2	
69	Ramachandran	089538F	55	1		12/6/2011	1	1	2	2	splenic abscess
70	Suresh Kumar	077640F	35	1		12/6/2011	1	1	1	2	
71	Seethalakshmi	085206F	50	2		12/6/2011	2	2	2	2	
72	Tamilarasi	085356F	22	2		12/6/2011	2	2	2	2	
73	Ma2haran	085052F	51	1	1	12/6/2011	1	1	1	2	MODS
74	Saroja	102924F	40	2		12/31/2011	1	1	1	2	
75	Kuppusamy	119154F	66	1	1	1/24/2012	1	1	1	2	
76	Kalaiselvi	124538F	45	2	1	1/24/2012	2	2	2	2	
77	Suguna	314974d	44	2		1/26/2012	2	2	2	2	
78	Sokkammal	119670F	65	2	2	1/31/2012	2	2	2	2	
79	Dilli Kumar	119629F	50	1	1	2/2/2012	1	1	1	2	
80	Shanmugam	324142F	64	1		10/21/2012	2	2	2	2	
81	Jayavel	316505F	55	1		10/10/2012	1	2	2	1	
82	Aravindh	543389D	20	1		9/28/2012	2	2	2	2	
83	Priya	279878F	26	2		8/30/2012	2	2	2	2	
84	Jayaraman	279920F	65	1		8/30/2012	1	1	1	2	
				1=male 2=female	1 = pos 2= neg		1=yes 2=no	1=yes 2=no	1=yes 2=no	1=yes 2=no	

S2	Occup.	Eschar present	2 of esc hars	siz	x	Macul opapularrash	Firstsite	dist (ant/post)	Secondsiteofeschar	durationo2fhospitalization	durationo2ffever	defervescenceoffever	contactwith	LNE	SiteofLNE	Place	ScrubtyphusIgMELISA
1	5	1	1	6	5	2	4	1		8	7	2	1	1		5	
2	1	1	1	3	2	2	6	1		5	10	1	1	3		5	1
3	1	1	1	8	4	2	4	1		4	10	3	1	2	1	5	1
4	2	1	1	15	5	2	1	1		5	10	2	1	1		5	1
5	4	1	1	10	7	2	7	2		8	10	1	1	1		5	1
6	2	1	1	15	10	2	4	1			15		1	1		5	
7	1	1	1	6	5	2	6	1			10		1	1		1	
8	5	1	1	4	4	2	4	1		9	21	5	1	2	2	2	1
9	2	1	1	5	3	2	5	1		8	10		1	1		5	1
10	1	1	1	4	2	2	1	1		3	7	1	1	1		4	1
11	1	1	1	6	5	2	4	2		7	7	2	1	1		5	1
12	9	1	1	7	6	2	6	1		7	15	3	1	1		4	1
13	2	1	1	4	3	1	4	1			7		1	1		2	1
14	2	1	1	6	5	2	4	1			15	2	1	2	2	1	1
15	1	1	1	5	4	2	3	1		6	15	1	1	2	2	5	1
16	2	1	1	4	3	2	4	2			10		1	1		4	
17	1	1	1	7	5	2	4	2			10		1	1		1	
18	1	1	1	7	4	2	2	1			6		1	3		1	
19	1	1	1	6	3	2	1	1		4	7	1	1	1		5	1
20	2	1	1	5	3	2	1	1		6	7	2	1	2	1	5	2
21	6	1	1	3	3	2	4	1		6	10	1	1	1		2	1
22	7	1	1	3	2	2	4	1			25		2	1		1	1
23	1	1	1	10	5	2	1	1		11	7	4	1	1		5	
24	1	1	1	15	8	2	2	1		10	5	1	1	1		5	1
25	1	1	1	3	3	2	1	1			7		1	1		5	1
26	1	1	1	10	6	2	6	1		4	12	1	1	3		5	1
27	1	2	0	0	0	2				5	7	1	1	1		5	1
28	2	1	1	4	3	2	7	1		14	10	1	1	1		1	1
29	8	2	0	0	0	2				6	10	1	1	3		1	1
30	1	1	1	8	6	2	7	1		10	7	2	1	1		5	1
31	2	2	0	0	0	2				5	12	1	1	1		2	1
32	2	2	0	0	0	2					7	1	1	1		5	1
33	2	1	1	8	6	2	5	1		5	12	2	1	1		1	1
34	1	1	1	6	5	2	4	1		6	10	2	1	1		5	1
35	2	1	1	6	6	2	4	1		9	7		1	1		4	1
36	1	1	1	9	5	2	6	1			7		1	1		5	1
37	1	1	1	5	3	2	6	1		4	10	1	1	1		5	
38	2	1	1	15	3	2	4	1		8	10	1	1	1		5	1
39	1	1	1	6	3	2	5	1		6	10	3	1	1		5	1
40	1	2	0	0	0	2				9	15	3	1	1		5	1

KEY - Occup. - 1=manual labourer, 2=housewife, 3=retired/unemployed, 4=office, 5=driver, 6=nurse, 7=salesman, 8=student, 9=misc; **First site and secondsiteofeschar** - 1=axilla, 2=groin, 3=genitalia, 4=trunk, 5=upper limb, 6=lower limb, 7=neck; **LNE-** 1=nil, 2=regional, 3= generalized; SiteofLNE – 1=axilla,2=inguinal; **Place** - 1=Chittoor, 2= Cuddapah, 3=Krishnagiri, 4=Tiruvannamalai, 5=Vellore; **Rash** and **contactwith** – 1=yes, 2=no

41	1	1	1	8	5	2	2	1		6	10	2	1	1		5	1
42	2	1	1	6	6	2	6	1			5		1	1		5	
43	1	1	1	5	3	2	4	1		3	7	2	1	1		5	1
44	1	1	1	5	3	2	6	1		11	15	1	1	2	2	5	1
45	2	1	1	5	5	2	4	2		9	5	5	1	1		5	1
46	1	1	1	5	4	2	4	2		7	14	2	1	1		5	1
47	1	1	1	3	3	2	4	1		25	5	4	1	2	1	5	1
48	2	1	1	7	3	2	4	1		4	8	1	1	1		5	
49	1	1	2	5	5	2	1	1	4	4	7	1	1	1		1	
50	2	1	1	5	4	2	6	1		3	10		1	1		4	1
51	1	1	1	4	3	2	4	1		6	15	1	1	1		1	1
52	1	1	1	5	3	2	4	1		2	15		1	1		5	
53	2	1	1	12	3	2	4	1		4	5	1	1	1		5	1
54	2	1	1	3	2	2	6	1		9	10	2	1	1		5	1
55	2	1	1	4	4	2	4	2		24	7	3	1	1		5	1
56	3	2	0	0	0	2				12	10		1	1		5	1
57	6	1	1	5	4	2	4	2		3	7	2	1	1		5	1
58	1	2	0	0	0	2				4	10	1	1	1		5	1
59	2	2	0	0	0	2				5	10	2	1	1		5	1
60	2	1	1	15	8	2	4	1		11	5	1	1	1		5	1
61	1	1	1	8	5	2	2	1		5	10	1	1	1		1	
62	1	1	1	5	2	2	3	1		8	10	2	1	1		5	1
63	1	1	1	5	5	2	6	1		11	7	3	1	2	2	4	1
64	7	1	1	5	5	2	5	1		3	20	1	1	1		5	
65	1	1	1	8	4	2	4	1		16	5	1	1	1		5	1
66	2	2	0	0	0	2				5	5	1	1	1		5	1
67	2	1	1	9	5	2	4	2		3	7		1	1		5	1
68	1	1	1	7	5	2	4	1		4	10	1	1	1		5	
69	1	1	1	5	4	2	3	1		9	10	1	1	2	2	5	1
70	1	2	0	0	0	2				11	15	3	1	1		5	1
71	1	2	0	0	0	2				14	5	2	1	1		4	1
72	4	2	0	0	0	2				4	9	2	1	1		5	1
73	1	1	1	8	4	2	4	1		9	10	2	1	1		5	1
74	1	1	1	6	5	2	1	1		5	7	2	1	2	1	5	
75	4	1	1	20	12	2	7	1		4	15	2	1	1		5	1
76	1	1	1	6	5	2	4	1			9		1	1		5	1
77	1	1	1	25	9	2	2	1		3	10	1	1	1		5	1
78	1	1	1	7	4	2	4	1		8	7	3	1	1		5	1
79	3	1	1	6	4	2	4	1		6	15		1	1		1	1
80	3	1	1	3	3	2	3	1		5	10	1	1	1		4	1
81	1	1	1	10	8	2	4	1		5	30	1	1	1		4	1
82	8	1	3	4	4	2	3	1			5	1	1	3		5	2
83	9	1	1	7	4	2	1	1		1	9	1	1	1		3	
84	3	1	1	10	5	2	2	1		13	7	2	1	1		5	1

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S2	Total WBC	N	E	M	L	B	Shifttoleft	Platelets	creatinine	TB	DB	SGOT	SGPT	vasculitis	typeofvasculitis	panniculitis	vasculopathy
1	18000	82	1	4	13	0	Missing	72000	2	3.3	1.4	123	67	1	1	2	2
2	8200	83		9	8		Missing	154000	1.4	1.4	0.3	55	54	1	1	2	2
3	15000	83	1	4	12		Missing	6000	2.4	0.5	0.2	85	29	1	3	4	2
4	3700	78		6	16		Missing	65000	1.2	5	4.2	276	74	2		2	2
5	9800	84		2	11		Missing	108000	1.4	2.3	0.9	304	115	1	1	2	2
6	11300	77	0	5	17	1	Missing	25000	1	0.5	0.2	110	76	1	3	4	2
7	6200	65		6	29		Missing	20000	1.2	1.2	0.3	48	16	1	1	1	2
8	25300	74		5	16		1	9000	1	1.3	0.2	101	44	1	1	3	2
9	12800	64		11	25		2	10000	2	0.4	0.2	84	71	1	3	2	2
10	10100	82		8	9		2	92000	2.2	0.5	0.2	91	60	Missing			Missing
11	6000	77		6	15		2	56000	1	0.8	0.2	123	30	1	1	3	2
12	14800	66		7	26	1	2	102000	1	0.7	0.2	57	83	2		2	1
13	7000	80	0	3	17	0	2	83000	1	0.5	0.1	132	148	1	1	4	2
14	13000	56	0	7	37	0	2	60000	1.1	1.8	0.2	240	118	Missing			Missing
15	12900	50	0	4	45	1	2	147000	1	0.6	0.2	83	103	Missing			Missing
16	6700	58	0	8	33	1	2	92000	1	0.7	0.2	138	186	1	1	2	2
17	6600	44	2	12	42	0	2	71000	1			32	13	2		2	2
18	6900	56	1	9	33	1	2	14000	1			146	91	Missing			Missing
19	15500	84	0	4	11	1	2	30000	3	0.7	0.2	118	27	1	1	3	2
20	5100	82	0	4	14	0	2	118000	0.8	0.6	0.1	150	99	2		2	1
21	5700	46	2	6	46	0	2	212000	0.7	0.5	0.1	78	48	Missing			Missing
22	7300	71	1	9	19	0	2	134000	1	0.6	0.2	84	82	2		2	1
23	18700	92	0	0	8	0	2	33000	1.5	0.5	0.2	44	16	Missing			Missing
24	8200	77	0	4	18	1	2	75000	1.4	1.4	1	255	74	Missing			Missing
25	8500	66	0	4	29	1	2	75000	1.2	2.5	2	229	190	Missing			Missing
26	22400	76	0	2	18	0	1	45000	1.3	1.6	1.2	180	47	Missing			Missing
27	7600	68	0	7	25	0	2	141000	1.4	0.6	0.2	33	39	Missing			Missing
28	20200	72	0	2	26	0	2	108000	1.2	0.7	0.2	62	11	Missing			Missing
29	13500	57	0	3	39	1	2	178000	1.2	0.5	0.2	70	75	Missing			Missing
30	24300	85	2	4	9	0	2	90000	1	4	3.8	331	78	Missing			Missing
31	2300	46	0	7	47	0	2	68000	1.3	0.7	0.5	368	103	Missing			Missing
32	7000	72	0	8	20	0	2	105000	1.5	0.8	0.2	83	33	Missing			Missing
33	10900	77	1	4	18	0	2	120000	1.2	0.7	0.2	114	51	2		2	1
34	9000	65	0	10	24	1	2	59000	1.1	6.3	5.5	349	288	1	1	2	2
35	16900	78	0	5	16	1	2	75000	1.3	0.9	0.3	93	50	1	2	2	2
36	11900	63	0	9	28	0	2	225000	1.4	0.7	0.2	108	88	1	3	4	2
37	20100	80	0	0	20	0	2	75000	1.4	0.8	0.2	156	77	Missing			Missing
38	12100	53	0	9	38	0	2	65000	0.8	0.3	0.1	30	26	2		4	1
39	6800	66	0	7	25	2	2	64000	8	3.1	2.7	336	222	1	1	2	2
40	19800	50	0	7	42	1	2	163000	0.6	2.7	2.5	378	319	Missing			Missing
41	14400	74	0	1	25	0	2	67000	1.9	4.9	2.8	173	126	1	1	2	2

KEY: **shifttoleft, vasculitis, vasculopathy** – 1=yes, 2=no; **typeofvasculitis** – 1=lymphocytic, 2=neutrophilic, 3=mixed; **panniculitis** – 1=both, 2=no, 3=septal, 4=lobular

42	11600	69	0	6	24	1	2	271000	0.9	0.8	0.2	29	48	1	1	4	2
43	7200	66		5	29		2	105000	1.2	1.4	0.1	117	81	Missing			Missing
44	8000	50			50		2	88000	0.9	0.9	0.2	141	56	Missing			Missing
45	6900	81	0	3	16	0	2	75000	1.8	1.4	0.8	112	40	1	1	2	2
46	18300	59	0	7	32	2	2	180000	1.6	2.6	2.4	156	89	1	1	2	2
47	21200	82	0	8	10	0	2	12000	3.5	3.2	2.2	498	208	Missing			Missing
48	6800	57	0	4	38	1	2	171000	0.9	0.4	0.1	111	70	1	1	2	2
49	19200	57	0	1	42	0	2	90000	1.3	3	2.6	163	50	1	1	2	2
50	8200	82	0	2	14	0	1	75000	1.2	4	2.8	215	70	1	3	2	2
51	10400	79	0	6	15	0	2	101000	0.9	2.6	0.9	162	146	Missing			Missing
52	10800	42	0	7	50	1	2	131000	1.4	0.6	0.4	118	86	2		2	2
53	15100	84	0	3	8	0	1	220000	1.1	3	2.5	147	49	1	1	2	2
54	10700	45	0	10	45	0	2	247000	2.2	1	0.2	69	115	1	1	2	2
55	11200	68	0	1	31	0	2	120000	2.1	1.2	0.2	94	33	Missing			Missing
56	4300	81	1	2	16	0	2	99000	3.4	3.2	0.4	47	18	Missing			Missing
57	6000	69	0	4	26	1	2	60000	1.1	0.4	0.2	65	68	Missing			Missing
58	9100	61	0	8	31	0	2	121000	0.9	0.7	0.2	145	101	Missing			Missing
59	20500	71	0	2	27	0	2	113000	1	0.9	0.2	62	62	Missing			Missing
60	18300	70	1	6	22	1	2	20000	1	1.1	0.2	213	56	1	1	2	2
61	9700	67	0	3	27	0	1	45000	1.5	2	1.8	85	74	1	1	2	2
62	10400	79	0	6	14	1	2	102000	1.2	0.8	0.2	69	58	1	1	2	2
63	15300	85	0	3	8	0	1	70000	1.5	2.6	0.3	125	42	Missing			Missing
64	7700	75	0	6	19	0	2	21000	1.7	1.7	0.2	135	14	Missing			Missing
65	12800	91	0	4	5	0	2	76000	1.6	5.5	1.5	72	58	1	2	4	2
66	13800	78	1	6	14	1	2	75000	1.6	2.1	0.4	151	26	Missing			Missing
67	6400	84	1	3	12	0	2	47000	0.9	0.4	0.2	123	121	1	3	2	2
68	15000	86	0	1	13	0	2	20000	1.3	0.9	0.2	64	44	Missing			Missing
69	15400	62	0	7	30	1	2	183000	1.3	2.2	1.5	87	48	Missing			Missing
70	5200	85	0	6	12	0	2	30000	0.8	0.7	0.2	131	29	Missing			Missing
71	14400	66	1	12	21	0	2	151000	2.1	4.2	4	86	52	Missing			Missing
72	6900	76	0	3	21	0	2	77000	0.9	0.5	0.2	165	181	Missing			Missing
73	13900	86	0	4	10	0	2	44000	4.8	3.1	2	190	64	2		2	1
74	10600	88	0	0	12	0	2	38000	0.7	4.1	3.9	108	58	Missing			Missing
75	13200	87	0	3	10	0	2	9000	1.2	0.9	0.4	145	82	Missing			Missing
76	9700	90	0	5	5	0	Missing	90000	1.4	0.6	0.2	72	37	Missing			Missing
77	10600	62		3	35	0	2	10000	1.4	0.5	0.2	32	17	Missing			Missing
78	9200	75	0	8	15	2	2	106000	1.4	0.9	0.5	182	118	Missing			Missing
79	9100	68	1	1	28	0	1	79000	1.1	2.8	1.8	123	103	Missing			Missing
80	12900	88	0	8	4	0	2	60000	1.2	4.9	4.2	137	36	Missing			Missing
81	15100	73	0	7	20	0	2	221000	1.3	0.7	0.4	89	100	2		2	1
82	6600	44	3	16	37	0	2	145000	1.13	0.6	0.2	73	30	1	1	2	2
83	5800	61	0	12	27	0	2	51000	1.2	0.5	0.2	47	33	2		1	1
84	7400	81	1	6	12	0	2	55000	2.8	2.5	1.4	43	36	1	1	2	2

KEY: **shifttoleft, vasculitis, vasculopathy** – 1=yes, 2=no; **typeofvasculitis** – 1=lymphocytic, 2=neutrophilic, 3=mixed; **panniculitis** – 1=both, 2=no, 3=septal, 4=lobular